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(54) ***GOSSYPIUM HIRSUTUM* PLANTS WITH INCREASED FIBER STRENGTH COMPRISING A FIBER STRENGTH ALLELE SPANNING THE GLUC1.1A GENE FROM *GOSSYPIUM BARBADENSE***

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(58) **Field of Classification Search**

USPC .......................................................... 800/281

See application file for complete search history.

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(57) **ABSTRACT**

This invention relates to the field of agriculture, more specifically to the use of molecular biology techniques to alter fiber-producing plants, particularly cotton plants, and/or accelerate breeding of such fiber-producing plants. Methods and means are provided to alter fiber qualities, such as increasing fiber strength. Methods are also provided to identify molecular markers associated with fiber strength in a population of cotton varieties and related progenitor plants.

**13 Claims, 20 Drawing Sheets**

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**Figure 1a**

| | | Putative TATA BOX |
|---|---|---|
| GhGLUC1.1A-gDNA | 2246 | aatccttttgaaaaaaaaac--ttccagagagagta**tataaat**ccagcagtaggcacaagaaacgagcaccagttattga |
| GhGLUC1.1A-cDNA | 1 | ------------------------------------------------------------------gcaccagttattga |
| GbGLUC1.1A-gDNA | | -------------------------------------------------------------------------------- |
| GbGLUC1.1A-cDNA | | -------------------------------------------------------------------------------- |
| GhGLUC1.1D-gDNA | 3206 | aatccttttgaaaaaaaaaaaattccagagagagtg**tataaat**ccagcagtgtgcacaagaaacgagcaccagttattga |
| GhGLUC1.1D-cDNA | 1 | ------------------------------------------------------------------gcaccagttattga |
| GbGLUC1.1D-gDNA | | -------------------------------------------------------------------------------- |
| GbGLUC1.1D-cDNA | | -------------------------------------------------------------------------------- |

| | | **Putative start codon** → **putative Exon1 for *GLUC1.1D*** |
|---|---|---|
| GhGLUC1.1A-gDNA | 2324 | ctttcctttgtaaaaaaaaaaagt-gctgagatcaagaaatatagtgaaatatgggtccaagattttctgggtttttaat |
| GhGLUC1.1A-cDNA | 15 | ctttcctttgtaaaaaaaaaaagt-gctgagatcaagaaatatagtgaaatatgggtccaagattttctgggtttttaat |
| GbGLUC1.1A-gDNA | 1 | -------------------------gctgagatcaagaaatatagtgaaatatgggtccaagattttctgggtttttaat |
| GbGLUC1.1A-cDNA | 1 | --------------------------------------aatatagtgaaatatgggtccaagattttctgggtttttaat |
| GhGLUC1.1D-gDNA | 3286 | cattcctttgtaaaaaaaaaaagaagctgagatcaagaaatatagtgaaat**atg**ggtccaacattttctgggtttttaat |
| GhGLUC1.1D-cDNA | 15 | cattcctttgtaaaaaaaaaaagaagctgagatcaagaaatatagtgaaat**atg**ggtccaacattttctgggtttttaat |
| GbGLUC1.1D-gDNA | 1 | -------------------------gctgagatcaagaaatatagtgaaat**atg**ggtccaacattttctgggtttttaat |
| GbGLUC1.1D-cDNA | 1 | -------------------------gctgagatcaagaaatatagtgaaat**atg**ggtccaacattttctgggtttttaat |

| | | *Putative stop codon* / **putative start codon** / → **putative Exon1 for *GLUC1.1A*** → putative intron for *GLUC1.1A and D* |
|---|---|---|
| GhGLUC1.1A-gDNA | 2403 | ctaagca**atg**ctgtttttaactcaactcctctctctaacag/gtaaaacaaacttctctacagtgattttacagtaaatat |
| GhGLUC1.1A-cDNA | 94 | ctaagca**atg**ctgtttttaactcaactcctctctctaacag--------------------------------------- |
| GbGLUC1.1A-gDNA | 56 | ctaagca**atg**ctgtttttaactcaactcctctctctaacag/gtaaaacaaacttctctacagtgattttacagtaaatat |
| GbGLUC1.1A-cDNA | 43 | ctaagca**atg**ctgtttttaactcaactcctctctctaacag--------------------------------------- |
| GhGLUC1.1D-gDNA | 3366 | ctcagcaatggtgtttttaactcaactcctctctctaacag/gtaaaacaaacttctctacagtgattttacggtaagtat |
| GhGLUC1.1D-cDNA | 95 | ctcagcaatggtgtttttaactcaactcctctctctaacag--------------------------------------- |
| GbGLUC1.1D-gDNA | 56 | ctcagcaatggtgtttttaactcaactcctctctctaacag/gtaaaacaaacttctctacagtgattttacggtaagtat |
| GbGLUC1.1D-cDNA | 56 | ctcagcaatggtgtttttaactcaactcctctctctaacag--------------------------------------- |

**Figure 1b**

```
                                                                                     putative Exon2 for GLUC1.1A&D
                                                                                                          →
GhGLUC1.1A-gDNA   2483 ggctttgaaaaatatacaacaaaacatttatcttcaatccattttaattactgatctactatatatgttgcag/atggccg
GhGLUC1.1A-cDNA    132 -------------------------------------------------------------------------atggccg
GbGLUC1.1A-gDNA    136 ggctttgaaaaatatacaacaaaacatttatcttcaatccattttaattactgatctactatatatgttgcag/atggccg
GbGLUC1.1A-cDNA     81 -------------------------------------------------------------------------atggccg
GhGLUC1.1D-gDNA   3446 ggctttgaaaaatatacaacaaaacattta------------------tactgatctaccatatatgttgcag/atggccg
GhGLUC1.1D-cDNA    133 -------------------------------------------------------------------------atggccg
GbGLUC1.1D-gDNA    136 ggctttgaaaaatatacaacaaaacattta------------------tactgatctaccatatatgttgcag/atggccg
GbGLUC1.1D-cDNA     94 -------------------------------------------------------------------------atggccg

GhGLUC1.1A-gDNA   2563 tgatattggtgtttgctatggtttgaacggcaacaatcttccatctccaggagatgttattaatcttttcaaaactagtg
GhGLUC1.1A-cDNA    142 tgatattggtgtttgctatggtttgaacggcaacaatcttccatctccaggagatgttattaatcttttcaaaactagtg
GbGLUC1.1A-gDNA    216 tgatattggtgtttgctatggtttgaacggcaacaatcttccatctccaggagatgttattaatcttttcaaaactagtg
GbGLUC1.1A-cDNA     91 tgatattggtgtttgctatggtttgaacggcaacaatcttccatctccaggagatgttattaatcttttcaaaactagtg
GhGLUC1.1D-gDNA   3508 tgatattggtgtttgctatggtttgaacggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtg
GhGLUC1.1D-cDNA    143 tgatattggtgtttgctatggtttgaacggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtg
GbGLUC1.1D-gDNA    198 tgatattggtgtttgctatggtttgaacggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtg
GbGLUC1.1D-cDNA    104 tgatattggtgtttgctatggtttgaacggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtg

GhGLUC1.1A-gDNA   2643 gcataaacaatatcaggctctaccagccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatg
GhGLUC1.1A-cDNA    222 gcataaacaatatcaggctctaccagccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatg
GbGLUC1.1A-gDNA    296 gcataaacaatatcaggctctaccagccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatg
GbGLUC1.1A-cDNA    171 gcataaacaatatcaggctctaccagccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatg
GhGLUC1.1D-gDNA   3588 gcataaacaatatcaggctctaccagccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatg
GhGLUC1.1D-cDNA    223 gcataaacaatatcaggctctaccagccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatg
GbGLUC1.1D-gDNA    278 gcataaacaatatcaggctctaccagtcttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatg
GbGLUC1.1D-cDNA    184 gcataaacaatatcaggctctaccagtcttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatg

GhGLUC1.1A-gDNA   2723 agtacgacaaacgaggacatacaaagcctcgcaacggatcaaa---------gtgcagccgatgcatgggttaacaccaa
GhGLUC1.1A-cDNA    302 agtacgacaaacgaggacatacaaagcctcgcaacggatcaaa---------gtgcagccgatgcatgggttaacaccaa
GbGLUC1.1A-gDNA    376 agtacgacaaacgaggacatacaaagcctcgcaacggatcaaactcatcaaagtgcagccgatgcatgggttaacaccaa
GbGLUC1.1A-cDNA    251 agtacgacaaacgaggacatacaaagcctcgcaacggatcaaactcatcaaagtgcagccgatgcatgggttaacaccaa
GhGLUC1.1D-gDNA   3668 ggtccgagaaacgaggacatacaaagcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaa
GhGLUC1.1D-cDNA    303 ggtccgagaaacgaggacatacaaagcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaa
GbGLUC1.1D-gDNA    358 ggtccgagaaacgaggacatacaaagcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaa
GbGLUC1.1D-cDNA    264 ggtccgagaaacgaggacatacaaagcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaa
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**Figure 1c**

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GhGLUC1.1A-gDNA   2794 catcgtcccttataaggaagatgttcaattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctctt
GhGLUC1.1A-cDNA    373 catcgtcccttataaggaagatgttcaattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctctt
GbGLUC1.1A-gDNA    456 catcgtcccttataaggaagatgttcaattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctctt
GbGLUC1.1A-cDNA    331 catcgtcccttataaggaagatgttcaattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctctt
GhGLUC1.1D-gDNA   3739 catcgtcccttataaggacgatgttcagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctctt
GhGLUC1.1D-cDNA    374 catcgtcccttataaggacgatgttcagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctctt
GbGLUC1.1D-gDNA    429 catcgtcccttataaggacgatgttcagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctctt
GbGLUC1.1D-cDNA    335 catcgtcccttataaggacgatgttcagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctctt

GhGLUC1.1A-gDNA   2874 acattcctggtgccatgaacaacataatgaactcgctggcctcatttgggctaggcacgacgaaggttacgaccgtggtc
GhGLUC1.1A-cDNA    453 acattcctggtgccatgaacaacataatgaactcgctggcctcatttgggctaggcacgacgaaggttacgaccgtggtc
GbGLUC1.1A-gDNA    536 acattcctggtgccatgaacaacataatgaactcgctcgcctcatttgggctaggcacgacgaaggttacgaccgtggtc
GbGLUC1.1A-cDNA    411 acattcctggtgccatgaacaacataatgaactcgctcgcctcatttgggctaggcacgacgaaggttacgaccgtggtc
GhGLUC1.1D-gDNA   3819 acattcctgatgccatgaacaacataatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtc
GhGLUC1.1D-cDNA    454 acattcctgatgccatgaacaacataatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtc
GbGLUC1.1D-gDNA    509 acattcctgatgccatgaacaacataatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtc
GbGLUC1.1D-cDNA    415 acattcctgatgccatgaacaacataatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtc

GhGLUC1.1A-gDNA   2954 ccgatgaatgccctaagtacctcgtaccctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtat
GhGLUC1.1A-cDNA    533 ccgatgaatgccctaagtacctcgtaccctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtat
GbGLUC1.1A-gDNA    616 ccgatgaatgccctaagtacctcgtaccctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtat
GbGLUC1.1A-cDNA    491 ccgatgaatgccctaagtacctcgtaccctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtat
GhGLUC1.1D-gDNA   3899 ccgatgaatgccctaagtacctcgtaccctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtat
GhGLUC1.1D-cDNA    534 ccgatgaatgccctaagtacctcgtaccctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtat
GbGLUC1.1D-gDNA    589 ccgatgaatgccctaagtacctcgtaccctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtat
GbGLUC1.1D-cDNA    495 ccgatgaatgccctaagtacctcgtaccctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtat
```

*putative premature stop codon for GbGLUC1.1A*

```
GhGLUC1.1A-gDNA   3034 catggccattctggttcgacaggattcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactc
GhGLUC1.1A-cDNA    613 catggccattctggttcgacaggattcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactc
GbGLUC1.1A-gDNA    696 catggccattctggtttgacaggattcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactc
GbGLUC1.1A-cDNA    571 catggccattctggtttgacaggattcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactc
GhGLUC1.1D-gDNA   3979 catggccattctggctgtacaggattcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactc
GhGLUC1.1D-cDNA    614 catggccattctggctgtacaggattcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactc
GbGLUC1.1D-gDNA    669 catggccattctggctgtacaggattcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactc
GbGLUC1.1D-cDNA    575 catggccattctggctgtacaggattcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactc
```

**Figure 1d**

| | | |
|---|---|---|
| GhGLUC1.1A-gDNA | 3114 | atatttccctcaactacgccttgttcacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctcttt |
| GhGLUC1.1A-cDNA | 693 | atatttccctcaactacgccttgttcacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctcttt |
| GbGLUC1.1A-gDNA | 776 | atatttccctcaactacgccttgttcacctcgaccgcaccggtggtggtcgaccaacgcttggaatactacaacctcttt |
| GbGLUC1.1A-cDNA | 651 | atatttccctcaactacgccttgttcacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctcttt |
| GhGLUC1.1D-gDNA | 4059 | atatttccctcgattacgccttgttcacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctcttt |
| GhGLUC1.1D-cDNA | 694 | atatttccctcgattacgccttgttcacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctcttt |
| GbGLUC1.1D-gDNA | 749 | atatttccctcgattacgccttgttcacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctcttt |
| GbGLUC1.1D-cDNA | 655 | atatttccctcgattacgccttgttcacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctcttt |
| | | |
| GhGLUC1.1A-gDNA | 3194 | gacggcatggtcgatgctttcaatgccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactgg |
| GhGLUC1.1A-cDNA | 773 | gacggcatggtcgatgctttcaatgccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactgg |
| GbGLUC1.1A-gDNA | 856 | gacggcatagtcgatgctttcaatgccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactgg |
| GbGLUC1.1A-cDNA | 731 | gacggcatagtcgatgctttcaatgccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactgg |
| GhGLUC1.1D-gDNA | 4139 | gacggcatggtcgatgctttcaatgccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactgg |
| GhGLUC1.1D-cDNA | 774 | gacggcatggtcgatgctttcaatgccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactgg |
| GbGLUC1.1D-gDNA | 829 | gacggcatggtcgatgctttcaatgccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactgg |
| GbGLUC1.1D-cDNA | 735 | gacggcatggtcgatgctttcaatgccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactgg |
| | | |
| GhGLUC1.1A-gDNA | 3274 | atggccgaccgccggtaacgagccttacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtga |
| GhGLUC1.1A-cDNA | 853 | atggccgaccgccggtaacgagccttacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtga |
| GbGLUC1.1A-gDNA | 936 | atggccgaccgccggtaacgagccttacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtga |
| GbGLUC1.1A-cDNA | 811 | atggccgaccgccggtaacgagccttacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtga |
| GhGLUC1.1D-gDNA | 4219 | atggccgaccgccggtaacgagccttacacgagtgtcgcgaacgctcaaacttataacaagaacttgttaaatcatgtga |
| GhGLUC1.1D-cDNA | 854 | atggccgaccgccggtaacgagccttacacgagtgtcgcgaacgctcaaacttataacaagaacttgttaaatcatgtga |
| GbGLUC1.1D-gDNA | 909 | atggccgaccgccggtaacgagccttacacgagtgtcgcgaacgctcaaacttataacaagaacttgttaaatcatgtga |
| GbGLUC1.1D-cDNA | 815 | atggccgaccgccggtaacgagccttacacgagtgtcgcgaacgctcaaacttataacaagaacttgttaaatcatgtga |
| | | |
| GhGLUC1.1A-gDNA | 3354 | cgcagaaagggactccgaaaagacctgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaa |
| GhGLUC1.1A-cDNA | 933 | cgcagaaagggactccgaaaagacctgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaa |
| GbGLUC1.1A-gDNA | 1016 | cgcagaaagggactccgaaaagacctgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaa |
| GbGLUC1.1A-cDNA | 891 | cgcagaaaggggctccgaaaagacctgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaa |
| GhGLUC1.1D-gDNA | 4299 | cgcagaaggggactccgaaaagacctgaatatataatgccgacgtttttcttcgagatgttcaacgaggatttgaagcaa |
| GhGLUC1.1D-cDNA | 934 | cgcagaaggggactccgaaaagacctgaatatataatgccgacgtttttcttcgagatgttcaacgaggatttgaagcaa |
| GbGLUC1.1D-gDNA | 989 | cgcagaaggggactccgaaaagacctgaatatataatgccgacgtttttcttcgagatgttcaacgaggatttgaagcaa |
| GbGLUC1.1D-cDNA | 895 | cgcagaaggggactccgaaaagacctgaatatataatgccgacgtttttcttcgagatgttcaacgaggatttgaagcaa |

**Figure 1e**

```
                                                                                                  putative stop codon for GLUC1.1A&D except GbGLUC1.1A
GhGLUC1.1A-gDNA    3434 cccacagttgagcagaatttcggattcttcttccccaatatgaaccctgtttatccattttggtgaacttgaaatgttat
GhGLUC1.1A-cDNA    1013 cccacagttgagcagaatttcggattcttcttccccaatatgaaccctgtttatccattttggtgaacttgaaatgttat
GbGLUC1.1A-gDNA    1096 cccacagttgagcagatgtt---------------caacgaga----tgttcaac-------gagaacttgaaatgttat
GbGLUC1.1A-cDNA     971 cccacagtagagcagatgtt---------------caacgaga----tgttcaac-------gagaacttgaaatgttat
GhGLUC1.1D-gDNA    4379 cccacagttgagcagaatttcggattcttcttccccaatatgaaccctgtttatccattttggtgaagttgaaatg---t
GhGLUC1.1D-cDNA    1014 cccacagttgagcagaatttcggattcttcttccccaatatgaaccctgtttatccattttggtgaagttgaaatg---t
GbGLUC1.1D-gDNA    1069 cccacagttgagcagaatttcggattcttcttccccaatatgaaccctgtttatccattttggtgaagttgaaatg---t
GbGLUC1.1D-cDNA     975 cccacagttgagcagaatttcggattcttcttccccaatatgaaccctgtttatccattttggtgaagttgaaatg---t

GhGLUC1.1A-gDNA    3514 tgttggctatttaaatcttttgccagagacgcttcatatagtttctgcatattttgaaagtggaaaatcaatctaaatat
GhGLUC1.1A-cDNA    1093 tgttggctatttaaatcttttgccagagacgcttcatatagtttctgcatattttgaaagtggaaa--------------
GbGLUC1.1A-gDNA    1150 tgttggctatttaaatcttttgccagagacgcttcatatag---------------------------------------
GbGLUC1.1A-cDNA    1025 tgttggctatttaaatcttttgccagagacgcttcatatag---------------------------------------
GhGLUC1.1D-gDNA    4456 tgttggctatttaaatcttttgccagagacgcttcatatagtttctgcatattttgaaagtggaaaatcaatctaaatat
GhGLUC1.1D-cDNA    1091 tgttggctatttaaatcttttgccagagacgcttcatatagtttctgcatattttgaaagtggaaaatcaatctaaatat
GbGLUC1.1D-gDNA    1146 tgttggctatttaaatcttttgccagagacgcttcatatag---------------------------------------
GbGLUC1.1D-cDNA    1052 tgttggctatttaaatcttttgccagagacgctccatatagtttctgcatattttgaaagtggaaagtcaatctaaatat

GhGLUC1.1A-gDNA    3594 aaataagttttatttgttgttttttaattaaataaaattttaaatattttaaaaacatctttattggtaattaaatatta
GhGLUC1.1A-cDNA    1159 -------------------------------------------------------------------------------a
GbGLUC1.1A-gDNA         --------------------------------------------------------------------------------
GbGLUC1.1A-cDNA         --------------------------------------------------------------------------------
GhGLUC1.1D-gDNA    4536 taataagttttatgtgttgttttttaattaaataaaattttaaatattttaaaaatatctttattggtaattaaatatta
GhGLUC1.1D-cDNA    1171 taataagttttatgtgttgttttttaattaaataaaattttaaatattataaaaa------------------------a
GbGLUC1.1D-gDNA         --------------------------------------------------------------------------------
GbGLUC1.1D-cDNA    1132 taataagttt--tgtgttgttttttaattaaataaaattttaaatattttggaaa------------------------a

GhGLUC1.1A-gDNA    3674 aataaaaagtttaatattcaaattttatcaattcaaaaataaaataaaaatatattaaatttatttttacgaataaattg
GhGLUC1.1A-cDNA    1160 aaaaaaaa--------------------------aaaaaaaaaaaaaaaaaa----------------------------
GbGLUC1.1A-gDNA         --------------------------------------------------------------------------------
GbGLUC1.1A-cDNA         --------------------------------------------------------------------------------
GhGLUC1.1D-gDNA    4616 aataaaaagtttaatattcaaattttatcaattcaaaaataaaataaaaatatattaaatttatttttacgaataaattg
GhGLUC1.1D-cDNA    1227 aaaaaaaa--------------------------aaaaaaaaaaaaaaaa------------------------------
GbGLUC1.1D-gDNA         --------------------------------------------------------------------------------
GbGLUC1.1D-cDNA    1186 aaaaaaaa--------------------------aaaaaaaaaaaaaaaa------------------------------
```

**Figure 2**

```
                         putative signal peptide   ><: Putative post-translational splicing site
GhGLUC1.1A       1 ------------mlfltqllsltdgr><digvcyglngnnlpspgdvinlfktsginnirlyqpypevleaargsgislsms
GbGLUC1.1A       1 ------------mlfltqilsltdgr><digvcyglngnnlpspgdvinlfktsginnirlyqpypevleaargsgislsms
GhGLUC1.1D       1 mgptfsgflisamvfltqilsltdgr><digvcyglngnnlpspgdvinlyktsginnirlyqpypevleaargsgislsmg
GbGLUC1.1D       1 mgptfsgflisamvfltqilsltdgr><digvcyglngnnlpspgdvinlyktsginnirlyqsypevleaargsgislsmg

GhGLUC1.1A      69 ttnediqslatdq---saadawvntnivpykedvqfrfiiigneaipgqsssyipgamnnimnslasfglgttkvttvvp
GbGLUC1.1A      69 ttnediqslatdqthqsaadawvntnivpykedvqfrfiiigneaipgqsssyipgamnnimnslasfglgttkvttvvp
GhGLUC1.1D      81 prnediqslakdq---saadawvntnivpykddvqfklitigneaisgqsssyipdamnnimnslalfglgttkvttvvp
GbGLUC1.1D      81 prnediqslakdq---saadawvntnivpykddvqfklitigneaisgqsssyipdamnnimnslalfglgttkvttvvp

GhGLUC1.1A     146 mnalstsyppsdgafgsditsimtsimailvrqdspllinvypyfayasdpthislnyalftstapvvvdqgleyynlfd
GbGLUC1.1A     149 mnalstsyppsdgafgsditsimtsimailv-------------------------------------------------
GhGLUC1.1D     158 mnalstsyppsdgafgsditsimtsimailavqdspllinvypyfayasdpthisldyalftstapvvvdqgleyynlfd
GbGLUC1.1D     158 mnalstsyppsdgafgsditsimtsimailavqdspllinvypyfayasdpthisldyalftstapvvvdqgleyynlfd

                                     GH17 signature
GhGLUC1.1A     226 gmvdafnaaldkigfgqitlivaetgwptagnepytsvanaqtynknllnhvtqkgtpkrpeyimptfffemfnenlkqp
GbGLUC1.1A         --------------------------------------------------------------------------------
GhGLUC1.1D     238 gmvdafnaaldkigfgqitlivaetgwptagnepytsvanaqtynknllnhvtqkgtpkrpeyimptfffemfnedlkqp
GbGLUC1.1D     238 gmvdafnaaldkigfgqitlivaetgwptagnepytsvanaqtynknllnhvtqkgtpkrpeyimptfffemfnedlkqp

GhGLUC1.1A     306 tveqnfgfffpnmnpvypfw
GbGLUC1.1A         --------------------
GhGLUC1.1D     318 tveqnfgfffpnmnpvypfw
GbGLUC1.1D     318 tveqnfgfffpnmnpvypfw
```

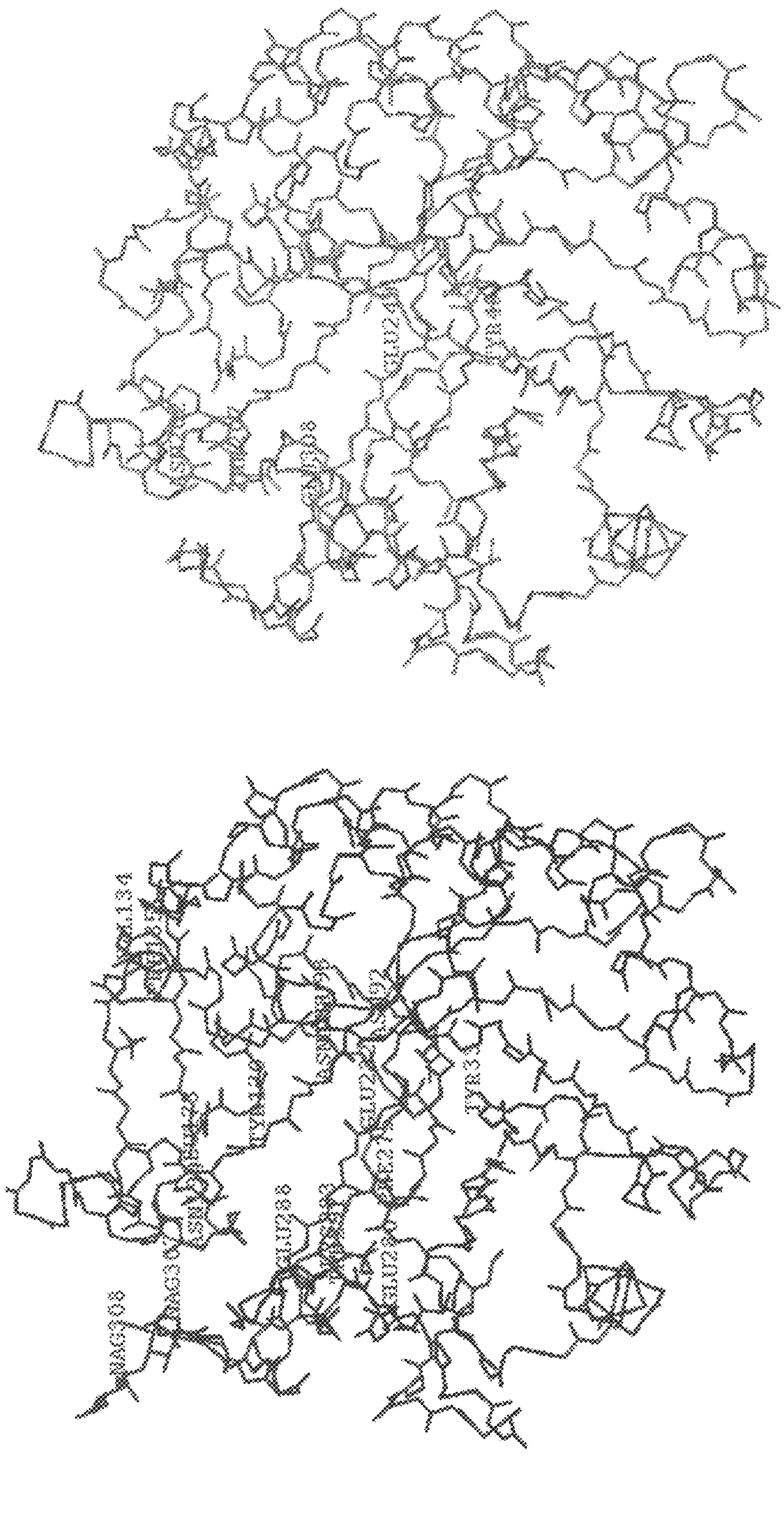
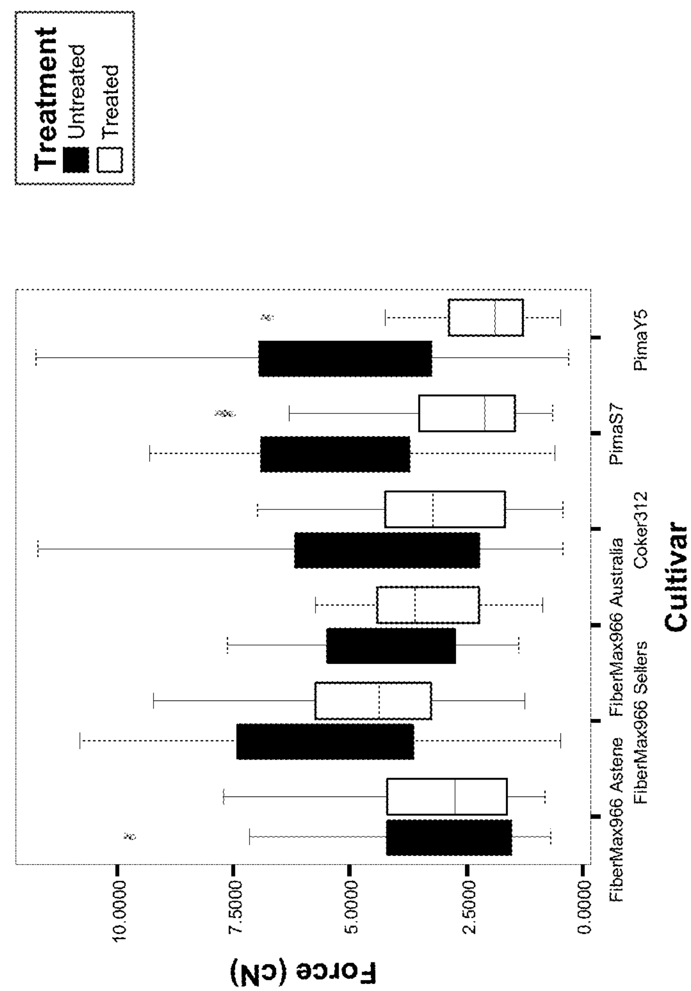


Figure 3a

Figure 3b

Figure 4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Untreated
Treated
Force (cN)
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7.5000
5.0000
2.5000
0.0000
FiberMax966 Astene
FiberMax966 Sellers
FiberMax966 Australia
Coker312
PimaS7
PimaY5
Cultivar

Figure 5

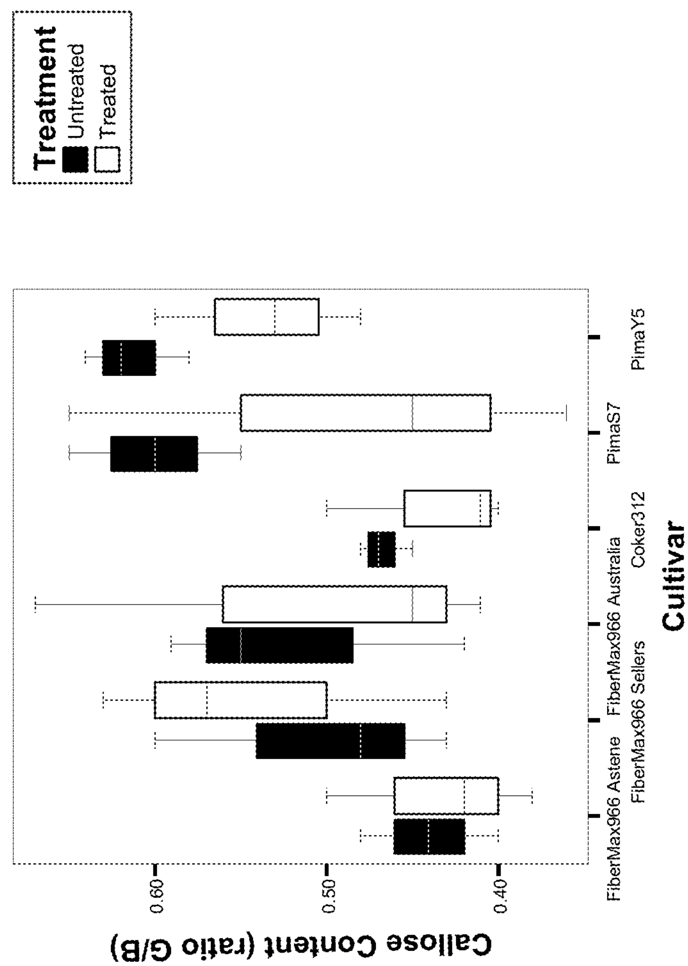

Treatment
Untreated
Treated
Callose Content (ratio G/B)
0.60
0.50
0.40
FiberMax966 Astene
FiberMax966 Sellers
FiberMax966 Australia
Coker312
PimaS7
PimaY5
Cultivar

**Figure 6a**

```
                                                                          Putative stop codon
                                          Putative start codon                Putative start codon
                      --------SE077--------→ → putative Exon1                  → putative Exon1
GhGLUC1.1A_gDNA      1 gctgagatcaagaaatatagtgaaatatgggtccaagattttctgggtttttaatctaagcaatgctgtttttaactcaa
GtGLUC1.1A-gDNA        --------------------------------------------------------------------------------
GbGLUC1.1A-gDNA      1 gctgagatcaagaaatatagtgaaatatgggtccaagattttctgggtttttaatctaagcaatgctgtttttaactcaa
GdGLUC1.1A-gDNA      1 gctgagatcaagaaatatagtgaaatatgggtccaagattttctgggtttttaatctaagcaatgctgtttttaactcaa
GmGLUC1.1A-gDNA        --------------------------------------------------------------------------------
GaGLUC1.1A-gDNA      1 gctgagatcaagaaatatagtgaaatatgggtccaagattttctgggtttttaatctcagcaatgctgtttttaactcaa
GheGLUC1.1A-gDNA     1 gctgagatcaagaaatatagtgaaatatgggtccaagattttctgggtttttaatctcagcaatgctgtttttaactcaa
GhGLUC1.1D_gDNA      1 gctgagatcaagaaatatagtgaaatatgggtccaaaattttctgggtttttaatctcagcaatggtgtttttaactcaa
GtGLUC1.1D-gDNA        --------------------------------------------------------------------------------
GbGLUC1.1D_gDNA      1 gctgagatcaagaaatatagtgaaatatgggtccaaaattttctgggtttttaatctcagcaatggtgtttttaactcaa
GdGLUC1.1D_gDNA      1 gctgagatcaagaaatatagtgaaatatgggtccaaaattttctgggtttttaatctcagcaatggtgtttttaactcaa
GmGLUC1.1D-gDNA        --------------------------------------------------------------------------------
GrGLUC1.1D-gDNA        --------------------------------------------------------------------------------

                              → putative intron1
GhGLUC1.1A_gDNA     81 ctcctctctctaacag/gtaaaacaaacttctctacagtgattttacagtaaatatggctttgaaaaatatacaacaaaac
GtGLUC1.1A-gDNA        ---------------------------------------------------------------------------------
GbGLUC1.1A-gDNA     81 ctcctctctctaacag/gtaaaacaaacttctctacagtgattttacagtaaatatggctttgaaaaatatacaacaaaac
GdGLUC1.1A-gDNA     81 ctcctctctctaacag/gtaaaacaaacttctctacagtgattttacagtaaatatggctttgaaaaatatacaacaaaac
GmGLUC1.1A-gDNA        ---------------------------------------------------------------------------------
GaGLUC1.1A-gDNA     81 ctcctctctctaacag/gtaaaacaaacttctctacagtgattttagagtaaatatggctttgaaaaatatacaacaaaac
GheGLUC1.1A-gDNA    81 ctcctctctctaacag/gtaaaacaaacttctctacagtgattttacagtaaatatggctttgaaaaatatacaacaaaac
GhGLUC1.1D_gDNA     81 ctcctctctctaacag/gtaaaacaaacttctctacagtgattttacggtaagtatggctttgaaaaatatacaacaaaac
GtGLUC1.1D-gDNA        ---------------------------------------------------------------------------------
GbGLUC1.1D_gDNA     81 ctcctctctctaacag/gtaaaacaaacttctctacagtgattttacggtaagtatggctttgaaaaatatacaacaaaac
GdGLUC1.1D_gDNA     81 ctcctctctctaacag/gtaaaacaaacttctctacagtgattttacggtaagtatggctttgaaaaatatacaacaaaac
GmGLUC1.1D-gDNA        ---------------------------------------------------------------------------------
GrGLUC1.1D-gDNA        ---------------------------------------------------------------------------------

                                                                → putative Exon2
GhGLUC1.1A_gDNA    161 atttatcttcaatccattttaattactgatctactatatatgttgcag/atggccgtgatattggtgtttgctatggtttg
GtGLUC1.1A-gDNA        ---------------------------------------------------------------------------------
GbGLUC1.1A-gDNA    161 atttatcttcaatccattttaattactgatctactatatatgttgcag/atggccgtgatattggtgtttgctatggtttg
GdGLUC1.1A-gDNA    161 atttatcttcaatccattttaattactgatctactatatatgttgcag/atggccgtgatattggtgtttgctatggtttg
GmGLUC1.1A-gDNA        ---------------------------------------------------------------------------------
GaGLUC1.1A-gDNA    161 atttatcttcaatccattttaattactgatctactatatatgttgcag/atggccgtgatattggtgtttgctatggtttg
GheGLUC1.1A-gDNA   161 atttatcttcaatccattttaattactgatctactatatatgttgcag/atggccgtgatattggtgtttgctatggtttg
GhGLUC1.1D_gDNA    161 atttatactgatctaccatatatgttgcag/atggccgtgatattggtgtttgctatggtttg
GtGLUC1.1D-gDNA        ---------------------------------------------------------------------------------
GbGLUC1.1D_gDNA    161 attta-------------------tactgatctaccatatatgttgcag/atggccgtgatattggtgtttgctatggtttg
GdGLUC1.1D_gDNA    161 attta-------------------tactgatctaccatatatgttgcag/atggccgtgatattggtgtttgctatggtttg
GmGLUC1.1D-gDNA        ---------------------------------------------------------------------------------
GrGLUC1.1D-gDNA        ---------------------------------------------------------------------------------
```

**Figure 6b**

```
                           --------SE003--------→
GhGLUC1.1A_gDNA     241 aacggcaacaatcttccatctccaggagatgttattaatcttttcaaaactagtggcataaacaatatcaggctctacca
GtGLUC1.1A-gDNA       1 --cggcaacaatcttccatctccaggagatgttattgatcttttcaaaactagtggcataaacaatatcaggctctacca
GbGLUC1.1A-gDNA     241 aacggcaacaatcttccatctccaggagatgttattaatcttttcaaaactagtggcataaacaatatcaggctctacca
GdGLUC1.1A-gDNA     241 aacggcaacaatcttccatctccaggagatgttattaatcttttcaaaactagtggcataaacaatatcaggctctacca
GmGLUC1.1A-gDNA       1 --cggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtggcataaacaatatcaggctctacca
GaGLUC1.1A-gDNA     241 aacggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtggcataaacaatatcaggctctacca
GheGLUC1.1A-gDNA    241 aacggcaacaatcttccatctccaggagatggtattaatctttacaaaactagtggcataaacaatatcaggctctacca
GhGLUC1.1D_gDNA     223 aacggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtggcataaacaatatcaggctctacca
GtGLUC1.1D-gDNA       1 --cggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtggcataaacaatatcaggctctacca
GbGLUC1.1D_gDNA     223 aacggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtggcataaacaatatcaggctctacca
GdGLUC1.1D_gDNA     223 aacggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtggcataaacaatatcaggctctacca
GmGLUC1.1D-gDNA       1 --cggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtggcataaacaatatcaggctctacca
GrGLUC1.1D-gDNA       1 --cggcaacaatcttccatctccaggagatgttattaatctttacaaaactagtggcataaacaatatcaggctctacca
          GLUC1.1D-SNP1 [c/t] GLUC1.1A-SNP7 [c/-]                        Putative premature stop codon
                        ↓     ↓                                          for GaGLUC1.1A
GhGLUC1.1A_gDNA     321 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgagtacgacaaacgaggacatacaaa
GtGLUC1.1A-gDNA      79 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgagtacgacaaacgaggacatacaaa
GbGLUC1.1A-gDNA     321 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgagtacgacaaacgaggacatacaaa
GdGLUC1.1A-gDNA     321 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgagtacgacaaacgaggacatacaaa
GmGLUC1.1A-gDNA      79 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgagtacgacaaacgaggacatacaaa
GaGLUC1.1A-gDNA     321 gccttac-ctgaagtgctcgaaggagcaaggggatcgggaatatccctctcgatgagtacgacaaacgaggacatacaaa
GheGLUC1.1A-gDNA    321 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgagtacgacaaacgaggacatacaaa
GhGLUC1.1D_gDNA     303 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgggtccgagaaacgaggacatacaaa
GtGLUC1.1D-gDNA      79 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgggtccgagaaacgaggacatacaaa
GbGLUC1.1D_gDNA     303 g-cttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgggtccgagaaacgaggacatacaaa
GdGLUC1.1D_gDNA     303 g-cttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgggtccgagaaacgaggacatacaaa
GmGLUC1.1D-gDNA      79 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgggtccgagaaacgaggacatacaaa
GrGLUC1.1D-gDNA      79 gccttaccctgaagtgctcgaagcagcaaggggatcgggaatatccctctcgatgggtccgagaaacgaggacatacaaa
                                    GLUC1.1A-SNP2 [---------/ctcatcaaa]
                                          ↓
GhGLUC1.1A_gDNA     401 gcctcgcaacggatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggaagatgtt
GtGLUC1.1A-gDNA     159 gcctcgcaacggatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggaagatgtt
GbGLUC1.1A-gDNA     401 gcctcgcaacggatcaaactcatcaaagtgcagccgatgcatgggttaacaccaacatcgtcccttataaggaagatgtt
GdGLUC1.1A-gDNA     401 gcctcgcaacggatcaaactcatcaaagtgcagccgatgcatgggttaacaccaacatcgtcccttataaggaagatgtt
GmGLUC1.1A-gDNA     159 gcctcgcaacggatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggaagatgtt
GaGLUC1.1A-gDNA     400 gcctcgcaacggatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggacgatgtt
GheGLUC1.1A-gDNA    401 gcctcgcaacggatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggacgatgtt
GhGLUC1.1D_gDNA     383 gcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggacgatgtt
GtGLUC1.1D-gDNA     159 gcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggacgatgtt
GbGLUC1.1D_gDNA     383 gcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggacgatgtt
GdGLUC1.1D_gDNA     383 gcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggacgatgtt
GmGLUC1.1D-gDNA     159 gcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggacgatgtt
GrGLUC1.1D-gDNA     159 gcctcgcaaaagatcaaa---------gtgcagccgatgcatgggttaacaccaacatcgtcccttataaggacgatgtt
```

**Figure 6c**

```
GhGLUC1.1A_gDNA     472 caattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctcttacattcctggtgccatgaacaacat
GtGLUC1.1A-gDNA     230 caattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctcttacattcctggtgccatgaacaacat
GbGLUC1.1A-gDNA     481 caattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctcttacattcctggtgccatgaacaacat
GdGLUC1.1A-gDNA     481 caattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctcttacattcctggtgccatgaacaacat
GmGLUC1.1A-gDNA     230 caattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctcttacattcctggtgccatgaacaacat
GaGLUC1.1A-gDNA     471 caattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctcttacattcctggtgccatgaacaacat
GheGLUC1.1A-gDNA    472 caattcaggttcatcatcattgggaatgaagccattccaggacagtcaagctcttacattcctggtgccatgaacaacat
GhGLUC1.1D_gDNA     454 cagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctcttacattcctgatgccatgaacaacat
GtGLUC1.1D-gDNA     230 cagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctcttacattcctgatgccatgaacaacat
GbGLUC1.1D_gDNA     454 cagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctcttacattcctgatgccatgaacaacat
GdGLUC1.1D_gDNA     454 cagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctcttacattcctgatgccatgaacaacat
GmGLUC1.1D-gDNA     230 cagttcaagttgatcactattgggaatgaagccatttcaggacaatcaagctcttacattcctgatgccatgaacaacat
GrGLUC1.1D-gDNA     230 cagttcaaattgatcactattgggaatgaagccatttcaggacaatcaagctcttacattcctgatgccatgaacaacat
                                GLUC1.1A-SNP3 [g/c]
                                      ↓
GhGLUC1.1A_gDNA     552 aatgaactcgctggcctcatttgggctaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GtGLUC1.1A-gDNA     310 aatgaactcgctggcctcatttgggctaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GbGLUC1.1A-gDNA     561 aatgaactcgctcgcctcatttgggctaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GdGLUC1.1A-gDNA     561 aatgaactcgctcgcctcatttgggctaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GmGLUC1.1A-gDNA     310 aatgaactcgctcgcctcatttgggctaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GaGLUC1.1A-gDNA     551 aatgaactcgctcgcctcatttgggctaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GheGLUC1.1A-gDNA    552 aatgaactcgctcgcctcatttgggctaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GhGLUC1.1D_gDNA     534 aatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GtGLUC1.1D-gDNA     310 aatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GbGLUC1.1D_gDNA     534 aatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GdGLUC1.1D_gDNA     534 aatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
GmGLUC1.1D-gDNA     310 aatgaactcgctcgccttatttgggttaggcacgacgaaggttacgaccgtggtcccgatgaatgccctaaatacctcgt
GrGLUC1.1D-gDNA     310 aatgaactcgctcgcctcatttgggttaggcacaacgaaggttacgaccgtggtcccgatgaatgccctaagtacctcgt
                                                                             Putative premature
                                                                           stopcodon for Gb&dGLUC1.1A
                                                                           with GLUC1.1A-SNP5 [c/t]
GhGLUC1.1A_gDNA     632 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggttcgacaggat
GtGLUC1.1A-gDNA     390 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggttcgacaggat
GbGLUC1.1A-gDNA     641 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggtttgacaggat
GdGLUC1.1A-gDNA     641 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggtttgacaggat
GmGLUC1.1A-gDNA     390 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggttcgacaggat
GaGLUC1.1A-gDNA     631 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggttcgacaggat
GheGLUC1.1A-gDNA    632 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggttcgacaggat
GhGLUC1.1D_gDNA     614 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggctgtacaggat
GtGLUC1.1D-gDNA     390 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggctgtacaggat
GbGLUC1.1D_gDNA     614 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggctgtacaggat
GdGLUC1.1D_gDNA     614 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggctgtacaggat
GmGLUC1.1D-gDNA     390 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggctgtacaggat
GrGLUC1.1D-gDNA     390 accctccttcagacggcgcttttggaagcgatataacatcgatcatgactagtatcatggccattctggctgtacaggat
```

**Figure 6d**

```
GhGLUC1.1A_gDNA      712 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcaactacgccttgtt
GtGLUC1.1A-gDNA      470 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcaactacgccttgtt
GbGLUC1.1A-gDNA      721 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcaactacgccttgtt
GdGLUC1.1A-gDNA      721 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcaactacgccttgtt
GmGLUC1.1A-gDNA      470 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcaactacgccttgtt
GaGLUC1.1A-gDNA      711 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcaactacgccttgtt
GheGLUC1.1A-gDNA     712 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcaactacgccttgtt
GhGLUC1.1D_gDNA      694 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcgattacgccttgtt
GtGLUC1.1D-gDNA      470 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcgattacgccttgtt
GbGLUC1.1D_gDNA      694 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcgattacgccttgtt
GdGLUC1.1D_gDNA      694 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcgattacgccttgtt
GmGLUC1.1D-gDNA      470 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcgattacgccttgtt
GrGLUC1.1D-gDNA      470 tcgcccctcctgatcaatgtgtacccttattttgcctatgcctcagaccccactcatatttccctcgattacgccttgtt
                                                  GLUC1.1A-SNP8 [c/g]            GLUC1.1A-SNP6 [g/a]
                                                          ↓                              ↓
GhGLUC1.1A_gDNA      792 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GtGLUC1.1A-gDNA      550 cacctcggccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GbGLUC1.1A-gDNA      801 cacctcgaccgcaccggtggtggtcgaccaacgcttggaatactacaacctctttgacggcatagtcgatgctttcaatg
GdGLUC1.1A-gDNA      801 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatagtcgatgctttcaatg
GmGLUC1.1A-gDNA      550 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GaGLUC1.1A-gDNA      791 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GheGLUC1.1A-gDNA     792 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GhGLUC1.1D_gDNA      774 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GtGLUC1.1D-gDNA      550 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GbGLUC1.1D_gDNA      774 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GdGLUC1.1D_gDNA      774 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GmGLUC1.1D-gDNA      550 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
GrGLUC1.1D-gDNA      550 cacctcgaccgcaccggtggtggtcgaccaaggcttggaatactacaacctctttgacggcatggtcgatgctttcaatg
                             ←--------SE002--------
GhGLUC1.1A_gDNA      872 ccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactggatggccgaccgccggtaacgagcct
GtGLUC1.1A-gDNA      630 ccgccctagataagatcggcttcggcc-----------------------------------------------------
GbGLUC1.1A-gDNA      881 ccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactggatggccgaccgccggtaacgagcct
GdGLUC1.1A-gDNA      881 ccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactggatggccgaccgccggtaacgagcct
GmGLUC1.1A-gDNA      630 ccgccctagataagatcggcttcggcc-----------------------------------------------------
GaGLUC1.1A-gDNA      871 ccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactggatggccgaccgccggtaacgagcct
GheGLUC1.1A-gDNA     872 ccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactggatggccgaccgccggtaacgagcct
GhGLUC1.1D_gDNA      854 ccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactggatggccgaccgccggtaacgagcct
GtGLUC1.1D-gDNA      630 ccgccctagataagatcggcttcggcc-----------------------------------------------------
GbGLUC1.1D_gDNA      854 ccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactggatggccgaccgccggtaacgagcct
GdGLUC1.1D_gDNA      854 ccgccctagataagatcggcttcggccaaattactctcattgtagccgaaactggatggccgaccgccggtaacgagcct
GmGLUC1.1D-gDNA      630 ccgctctagataagatcggcttcggcc-----------------------------------------------------
GrGLUC1.1D-gDNA      630 ccgccctagataagatcggcttcggcc-----------------------------------------------------
```

**Figure 6e**

```
GhGLUC1.1A_gDNA     952  tacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtgacgcagaaagggactccgaaaagacc
GtGLUC1.1A-gDNA          --------------------------------------------------------------------------------
GbGLUC1.1A-gDNA     961  tacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtgacgcagaaagggactccgaaaagacc
GdGLUC1.1A-gDNA     961  tacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtgacgcagaaagggactccgaaaagacc
GmGLUC1.1A-gDNA          --------------------------------------------------------------------------------
GaGLUC1.1A-gDNA     951  tacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtgacgcagaaagggactccgaaaagacc
GheGLUC1.1A-gDNA    952  tacacgagtgtcgcgaacgctcaaacttataacaagaacttgttgaatcatgtgacgcagaaagggactccgaaaagacc
GhGLUC1.1D_gDNA     934  tacacgagtgtcgcgaacgctcaaacttataacaagaacttgttaaatcatgtgacgcagaaggggactccgaaaagacc
GtGLUC1.1D-gDNA          --------------------------------------------------------------------------------
GbGLUC1.1D_gDNA     934  tacacgagtgtcgcgaacgctcaaacttataacaagaacttgttaaatcatgtgacgcagaaggggactccgaaaagacc
GdGLUC1.1D_gDNA     934  tacacgagtgtcgcgaacgctcaaacttataacaagaacttgttaaatcatgtgacgcagaaggggactccgaaaagacc
GmGLUC1.1D-gDNA          --------------------------------------------------------------------------------
GrGLUC1.1D-gDNA          --------------------------------------------------------------------------------

GhGLUC1.1A_gDNA    1032  tgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaacccacagttgagcagaatttcggat
GtGLUC1.1A-gDNA          --------------------------------------------------------------------------------
GbGLUC1.1A-gDNA    1041  tgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaacccacagttgagcaga---------
GdGLUC1.1A-gDNA    1041  tgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaacccacagttgagcaga---------
GmGLUC1.1A-gDNA          --------------------------------------------------------------------------------
GaGLUC1.1A-gDNA    1031  tgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaacccacagttgagcagaatttcggat
GheGLUC1.1A-gDNA   1032  tgaatatataatgccgacgtttttcttcgagatgttcaacgagaacttgaagcaacccacagttgagcagaatttcggat
GhGLUC1.1D_gDNA    1014  tgaatatataatgccgacgtttttcttcgagatgttcaacgaggatttgaagcaacccacagttgagcagaatttcggat
GtGLUC1.1D-gDNA          --------------------------------------------------------------------------------
GbGLUC1.1D_gDNA    1014  tgaatatataatgccgacgtttttcttcgagatgttcaacgaggatttgaagcaacccacagttgagcagaatttcggat
GdGLUC1.1D_gDNA    1014  tgaatatataatgccgacgtttttcttcgagatgttcaacgaggatttgaagcaacccacagttgagcagaatttcggat
GmGLUC1.1D-gDNA          --------------------------------------------------------------------------------
GrGLUC1.1D-gDNA          --------------------------------------------------------------------------------

                                                                 Putative stop codon                  <---
GhGLUC1.1A_gDNA    1112  tcttcttccccaatatgaaccctgtttatccattttggtgaacttgaaatgttattgttggctatttaaatcttttgcca
GtGLUC1.1A-gDNA          --------------------------------------------------------------------------------
GbGLUC1.1A-gDNA    1112  ---tgttcaacgaga-------tgttcaac-------gagaacttgaaatgttattgttggctatttaaatcttttgcca
GdGLUC1.1A-gDNA    1112  ---tgttcaacgaga-------tgttcaac-------gagaacttgaaatgttattgttggctatttaaatcttttgcca
GmGLUC1.1A-gDNA          --------------------------------------------------------------------------------
GaGLUC1.1A-gDNA    1111  tcttcttccccaatatgaaccctgtttatccattttggtgaacttgaaatgttattgttggctatttaaatcttttgcca
GheGLUC1.1A-gDNA   1112  tcttcttccccaatatgaaccctgtttatccattttggtgagcttgaaatgttattgttggctatttaaatcttttgcca
GhGLUC1.1D_gDNA    1094  tcttcttccccaatatgaaccctgtttatccattttggtgaagttgaaatg---ttgttggctatttaaatcttttgcca
GtGLUC1.1D-gDNA          --------------------------------------------------------------------------------
GbGLUC1.1D_gDNA    1094  tcttcttccccaatatgaaccctgtttatccattttggtgaagttgaaatg---ttgttggctatttaaatcttttgcca
GdGLUC1.1D_gDNA    1094  tcttcttccccaatatgaaccctgtttatccattttggtgaagttgaaatg---ttgttggctatttaaatcttttgcca
GmGLUC1.1D-gDNA          --------------------------------------------------------------------------------
GrGLUC1.1D-gDNA          --------------------------------------------------------------------------------
```

**Figure 6f**

```
                              ←-----SE078-----
GhGLUC1.1A_gDNA     1192  gagacgcttcatatag
GtGLUC1.1A-gDNA           ----------------
GbGLUC1.1A-gDNA     1175  gagacgcttca-----
GdGLUC1.1A-gDNA     1175  gagacgcttca-----
GmGLUC1.1A-gDNA           ----------------
GaGLUC1.1A-gDNA     1191  gagacgcttcatatag
GheGLUC1.1A-gDNA    1192  gagacgcttcatatag
GhGLUC1.1D_gDNA     1171  gagacgcttcatatag
GtGLUC1.1D-gDNA           ----------------
GbGLUC1.1D_gDNA     1171  gagacgcttcatatag
GdGLUC1.1D_gDNA     1171  gagacgcttcatatag
GmGLUC1.1D-gDNA           ----------------
GrGLUC1.1D-gDNA           ----------------
```

**Figure 7a**

```
                     putative signal peptide   ><: Putative post-translational splicing site
GhGLUC1.1A-prot    1 ------------mlfltqllsltdgr><digvcyglngnnipspgdvinlfktsginnirlyqpypevleaargsgislsms
GtGLUC1.1A-prot    1 -------------------------------------gnnipspgdvidlfktsginnirlyqpypevleaargsgislsms
GbGLUC1.1A-prot    1 ------------mlfltqllsltdgr><digvcyglngnnipspgdvinlfktsginnirlyqpypevleaargsgislsms
GdGLUC1.1A-prot    1 ------------mlfltqllsltdgr><digvcyglngnnipspgdvinlfktsginnirlyqpypevleaargsgislsms
GmGLUC1.1A-prot    1 -------------------------------------gnnipspgdvinlyktsginnirlyqpypevleaargsgislsms
GaGLUC1.1A-prot    1 mgprfsgflisamlfltqllsltdgr><digvcyglngnnipspgdvinlyktsginnirlyqpyikcskeggdreypsr--
GheGLUC1.1A-prot   1 mgprfsgflisamlfltqllsltdgr><digvcyglngnnipspgdainlyktsginnirlyqpypevleaargsgislsms
GhGLUC1.1D-prot    1 mgprfsgflisamvfltqllsltdgr><digvcyglngnnipspgdvinlyktsginnirlyqpypevleaargsgislsmg
GtGLUC1.1D-prot    1 -------------------------------------gnnipspgdvinlyktsginnirlyqpypevleaargsgislsmg
GbGLUC1.1D-prot    1 mgprfsgflisamvfltqllsltdgr><digvcyglngnnipspgdvinlyktsginnirlyqsypevleaargsgislsmg
GdGLUC1.1D-prot    1 mgprfsgflisamvfltqllsltdgr><digvcyglngnnipspgdvinlyktsginnirlyqsypevleaargsgislsmg
GmGLUC1.1D-prot    1 -------------------------------------gnnipspgdvinlyktsginnirlyqpypevleaargsgislsmg
GrGLUC1.1D-prot    1 -------------------------------------gnnipspgdvinlyktsginnirlyqpypevleaargsgislsmg

GhGLUC1.1A-prot   69 ttnediqslatd---qsaadawvntnivpykedvqfrfiiigneaipgqsssyipgamnnimnslasfglgttkvttvvp
GtGLUC1.1A-prot   46 ttnediqslatd---qsaadawvntnivpykedvqfrfiiigneaipgqsssyipgamnnimnslasfglgttkvttvvp
GbGLUC1.1A-prot   69 ttnediqslatdgthqsaadawvntnivpykedvqfrfiiigneaipgqsssyipgamnnimnslasfglgttkvttvvp
GdGLUC1.1A-prot   69 ttnediqslatdgthqsaadawvntnivpykedvqfrfiiigneaipgqsssyipgamnnimnslasfglgttkvttvvp
GmGLUC1.1A-prot   46 ttnediqslatd---qsaadawvntnivpykedvqfrfiiigneaipgqsssyipgamnnimnslasfglgttkvttvvp
GaGLUC1.1A-prot       --------------------------------------------------------------------------------
GheGLUC1.1A-prot  81 ttnediqslatd---qsaadawvntnivpykddvqfrfiiigneaipgqsssyipgamnnimnslasfglgttkvttvvp
GhGLUC1.1D-prot   81 prnediqslakd---qsaadawvntnivpykddvqfklitigneaisgqsssyipdamnnimnslatfglgttkvttvvp
GtGLUC1.1D-prot   46 prnediqslakd---qsaadawvntnivpykddvqfklitigneaisgqsssyipdamnnimnslatfglgttkvttvvp
GbGLUC1.1D-prot   81 prnediqslakd---qsaadawvntnivpykddvqfklitigneaisgqsssyipdamnnimnslatfglgttkvttvvp
GdGLUC1.1D-prot   81 prnediqslakd---qsaadawvntnivpykddvqfklitigneaisgqsssyipdamnnimnslatfglgttkvttvvp
GmGLUC1.1D-prot   46 prnediqslakd---qsaadawvntnivpykddvqfklitigneaisgqsssyipdamnnimnslatfglgttkvttvvp
GrGLUC1.1D-prot   46 prnediqslakd---qsaadawvntnivpykddvqfklitigneaisgqsssyipdamnnimnslasfglgttkvttvvp

GhGLUC1.1A-prot  146 mnalstsyppsdgafgsditsimtsimailvrqdspllinvypyfayasdpthislnyalftstapvvvdqgleyynlfd
GtGLUC1.1A-prot  123 mnalstsyppsdgafgsditsimtsimailvrqdspllinvypyfayasdpthislnyalftssapvvvdqgleyynlfd
GbGLUC1.1A-prot  149 mnalstsyppsdgafgsditsimtsimailv-------------------------------------------------
GdGLUC1.1A-prot  149 mnalstsyppsdgafgsditsimtsimailv-------------------------------------------------
GmGLUC1.1A-prot  123 mnalstsyppsdgafgsditsimtsimailvrqdspllinvypyfayasdpthislnyalftstapvvvdqgleyynlfd
GaGLUC1.1A-prot   69 --------------------------------------------------------------------------------
GheGLUC1.1A-prot 158 mnalstsyppsdgafgsditsimtsimailvrqdspllinvypyfayasdpthislnyalftstapvvvdqgleyynlfd
GhGLUC1.1D-prot  158 mnalstsyppsdgafgsditsimtsimailavqdspllinvypyfayasdpthisldyalftstapvvvdqgleyynlfd
GtGLUC1.1D-prot  123 mnalstsyppsdgafgsditsimtsimailavqdspllinvypyfayasdpthisldyalftstapvvvdqgleyynlfd
GbGLUC1.1D-prot  158 mnalstsyppsdgafgsditsimtsimailavqdspllinvypyfayasdpthisldyalftstapvvvdqgleyynlfd
GdGLUC1.1D-prot  158 mnalstsyppsdgafgsditsimtsimailavqdspllinvypyfayasdpthisldyalftstapvvvdqgleyynlfd
GmGLUC1.1D-prot  123 mnalntsyppsdgafgsditsimtsimailavqdspllinvypyfayasdpthisldyalftstapvvvdqgleyynlfd
GrGLUC1.1D-prot  123 mnalstsyppsdgafgsditsimtsimailavqdspllinvypyfayasdpthisldyalftstapvvvdqgleyynlfd
```

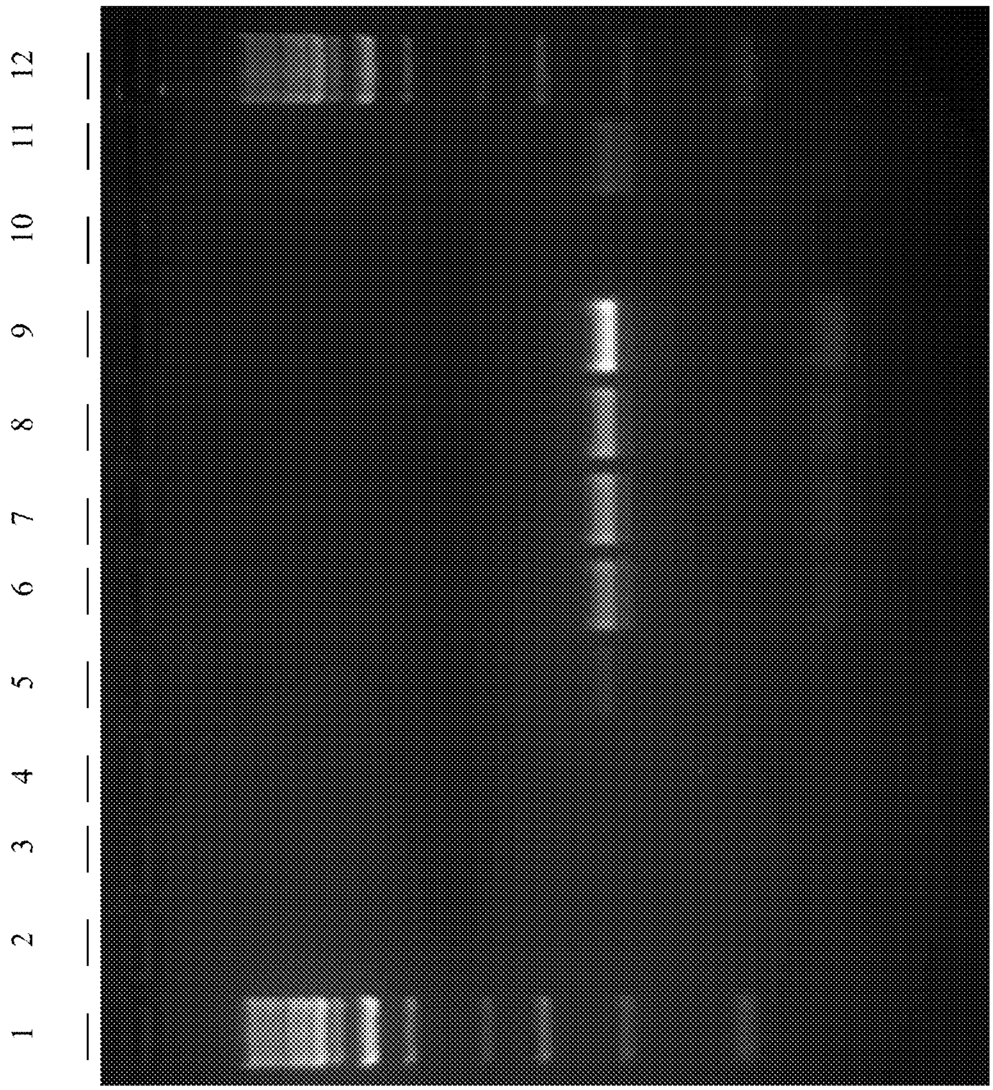
**Figure 7b**

**GH17 signature**

| | | |
|---|---|---|
| GhGLUC1.1A-prot | 226 | gmvdafnaaldkigfgq**itlivaetgwptag**nepytsvanaqtynknllnhvtqkgtpkrpeyimptfffemfnenlkqp |
| GtGLUC1.1A-prot | 203 | gmvdafnaaldkigfg---------------------------------------------------------------- |
| GbGLUC1.1A-prot | | -------------------------------------------------------------------------------- |
| GdGLUC1.1A-prot | | -------------------------------------------------------------------------------- |
| GmGLUC1.1A-prot | 203 | gmvdafnaaldkigfg---------------------------------------------------------------- |
| GaGLUC1.1A-prot | 76 | -------------------------------------------------------------------------------- |
| GheGLUC1.1A-prot | 238 | gmvdafnaaldkigfgq**itlivaetgwptag**nepytsvanaqtynknllnhvtqkgtpkrpeyimptfffemfnenlkqp |
| GhGLUC1.1D-prot | 238 | gmvdafnaaldkigfgq**itlivaetgwptag**nepytsvanaqtynknllnhvtqkgtpkrpeyimptfffemfnedlkqp |
| GtGLUC1.1D-prot | 203 | gmvdafnaaldkigfg---------------------------------------------------------------- |
| GbGLUC1.1D-prot | 238 | gmvdafnaaldkigfgq**itlivaetgwptag**nepytsvanaqtynknllnhvtqkgtpkrpeyimptfffemfnedlkqp |
| GdGLUC1.1D-prot | 238 | gmvdafnaaldkigfgq**itlivaetgwptag**nepytsvanaqtynknllnhvtqkgtpkrpeyimptfffemfnedlkqp |
| GmGLUC1.1D-prot | 203 | gmvdafnaaldkigfg---------------------------------------------------------------- |
| GrGLUC1.1D-prot | 203 | gmvdafnaaldkigfg---------------------------------------------------------------- |

| | | |
|---|---|---|
| GhGLUC1.1A-prot | 306 | tveqnfgfffpnmnpvypfw |
| GtGLUC1.1A-prot | | -------------------- |
| GbGLUC1.1A-prot | | -------------------- |
| GdGLUC1.1A-prot | | -------------------- |
| GmGLUC1.1A-prot | | -------------------- |
| GaGLUC1.1A-prot | | -------------------- |
| GheGLUC1.1A-prot | 318 | tveqnfgfffpnmnpvypfw |
| GhGLUC1.1D-prot | 318 | tveqnfgfffpnmnpvypfw |
| GtGLUC1.1D-prot | | -------------------- |
| GbGLUC1.1D-prot | 318 | tveqnfgfffpnmnpvypfw |
| GdGLUC1.1D-prot | 318 | tveqnfgfffpnmnpvypfw |
| GmGLUC1.1D-prot | | -------------------- |
| GrGLUC1.1D-prot | | -------------------- |

Figure 8



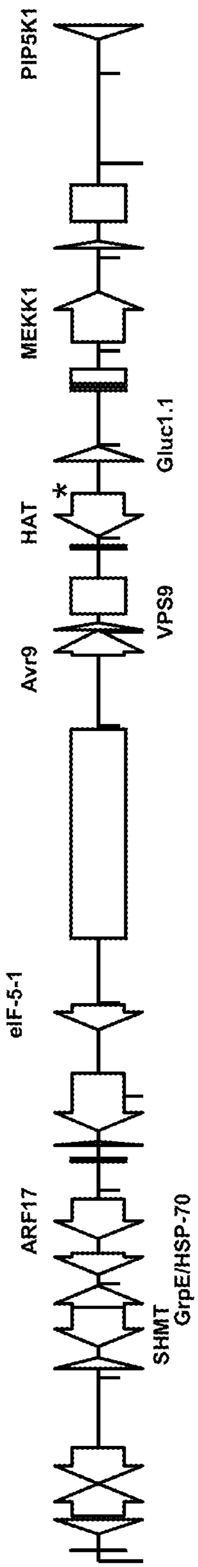

PIP5K1
MEKK1
Gluc1.1
HAT
VPS9
Avr9
eIF-5-1
ARF17
GrpE/HSP-70
SHMT

Figure 9

# *GOSSYPIUM HIRSUTUM* PLANTS WITH INCREASED FIBER STRENGTH COMPRISING A FIBER STRENGTH ALLELE SPANNING THE GLUC1.1A GENE FROM *GOSSYPIUM BARBADENSE*

## CROSS REFERENCE TO RELATED APPLICATION

This application is a §371 U.S. National Stage of International Application No. PCT/EP09/003674, which claims the benefit of U.S. Provisional Application Ser. No. 61/128,938, filed May 27, 2008, the contents of which are herein incorporated by reference in their entirety.

## REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The official copy of the sequence listing is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file named "CS9046PCTUSSequenceListingST25.txt", created on Nov. 11, 2010, and having a size of 358 kilobytes and is filed concurrently with the specification. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

## FIELD OF THE INVENTION

This invention relates to the field of agriculture, more specifically to the use of molecular biology techniques to alter fiber-producing plants, particularly cotton plants, and/or accelerate breeding of such fiber-producing plants. Methods and means are provided to alter fiber qualities, such as increasing fiber strength. Methods are also provided to identify molecular markers associated with fiber strength in a population of cotton varieties and related progenitor plants.

## BACKGROUND OF THE INVENTION

Cotton provides much of the high quality fiber for the textile industry. The modification of cotton fiber characteristics to better suit the requirements of the industry is a major effort in breeding by either classical methods or by genetically altering the genome of cotton plants.

About 90% of cotton grown worldwide is *Gossypium hirsutum* L., whereas *Gossypium barbadense* accounts for about 8%. As in most flowering plants, cotton genomes are thought to have incurred one or more polyploidization events and to have evolved by the joining of divergent genomes in a common nucleus. The cotton commerce is dominated by improved forms of two "AD" allotetraploid species, *Gossypium hirsutum* L. and *Gossypium barbadense* L (both 2n=4x=52). Allotetraploid cottons are thought to have formed about 1-2 million years ago, in the New World, by hybridization between a maternal Old World "A" genome taxon resembling *Gossypium herbaceum* (2n=2x=26) and paternal New World "D" genome taxon resembling *Gossypium raimondii* or *Gossypium gossypioides* (both 2n=2x=26). Wild A genome diploid and AD allotetraploid *Gossypium* taxa produce spinnable fibers. One A genome diploid species, *Gossypium arboreum* (2n=2x=26), remains intensively bred and cultivated in Asia. Its close relative and possible *Gossypium* progenitor, the A genome diploid species *G. herbaceum*, also produces spinnable fiber. Although the seeds of D genome diploids are pubescent, none produce spinnable fibers. No taxa from the other recognized diploid *Gossypium* genomes (B, C, E, F, G and K) have been domesticated. Intense directional selection by humans has consistently produced AD allotetraploid cottons that have superior yield and/or quality characteristics compared to the A genome diploid cultivars. Selective breeding of *G. hirsutum* (AADD; "Upland" cotton) has emphasized maximum yield, whereas *G. barbadense* (AADD; "Sea Island", "Pima", or "Egyptian" cotton) is prized for its fibers of superior length, strength, and fineness (Jiang et al., 1998, Proc Natl Acad Sci USA. 95(8): 4419-4424).

A cotton fiber is a single cell that initiates from the epidermis of the outer integument of the ovules, at or just prior to anthesis. Thereafter, the fibers elongate rapidly for about 3 weeks before they switch to intensive secondary cell wall cellulose synthesis. Fiber cells interconnect only to the underlying seed coat at their basal ends and influx of solute, water and other molecules occurs through either plasmodesmata or plasma membrane. Ruan et al. 2001 (Plant Cell 13: 47-63) demonstrated a transient closure of plasmodesmata during fiber elongation. Ruan et al. 2004 (Plant Physiology 136: 4104-4113) compared the duration of plasmodesmata closure among different cotton genotypes differing in fiber length and found a positive correlation between the duration of the plasmodesmata closure and fiber length. Furthermore, microscopic evidence was presented showing callose deposition and degradation at the fiber base, correlating with the timing of plasmodesmata closure and reopening. Expression of a endo-1,3-beta-glucanase gene in the fibers, allowing to degrade callose, correlated with the reopening of the plasmodesmata at the fiber base.

WO2005/017157 describes methods and means for modulating fiber length in fiber producing plants such as cotton by altering the fiber elongation phase. The fiber elongation phase may be increased or decreased by interfering with callose deposition in plasmodesmata at the base of the fiber cells.

WO2008/083969 (claiming priority of European patent application EP 07000550) discloses isolated DNA molecules comprising a nucleotide sequence encoding cotton endo-1,3-beta-glucanases and fiber cell preferential promoter or promoter regions, as well as methods for modifying the length of a fiber of a cotton plant using these sequences or promoters. WO2008/083969 also describes that the timing of expression of the A and D subgenome specific alleles of the fiber specific endo-1,3-beta-glucanase gene in *Gossypium hirsutum* is different. Whereas the onset of the expression of the D subgenome specific allele correlates with the end of the rapid elongation phase (about 14 to 17 days post-anthesis, hereinafter abbreviated "DPA"), onset of the expression of the A subgenome specific allele is delayed until the beginning of the late fiber maturation phase (about 35-40 DPA) depending on growth conditions.

One fiber characteristic that is of special interest for the cotton industry is fiber strength. There is not only a high correlation between fiber strength and yarn strength, but also cotton with high fiber strength is more likely to withstand breakage during the manufacturing process.

Fiber strength is, among many other textile properties of cotton fibers (e.g., fiber wall thickness or maturity, dyeability, extensibility . . . ), described to be directly dependent on the amount and properties (e.g., degree of polymerization, crystallite size, and microfibril orientation) of cellulose (Ramey, 1986, In: Mauney J. R. and Stewart J. McD. (eds.) *Cotton Physiology. The Cotton Foundation*, Memphis, Tenn., pp. 351-360; Triplett, 1993, In: *Cellulosics: Pulp, Fibre, and Environmental Aspects*. Ellis Horwood, Chichester, UK, pp. 135-140; Hsieh, 1999, In: Basra A. S. (ed.) *Cotton Fibers: Developmental Biology, Quality Improvement, and Textile*

*Processing*. The Haworth Press, New York, pp. 137-166). Advances in the past decade, particularly using the model plant *Arabidopsis* (Arioli et al., 1998, Science 279(5351): 717-720), have led to a great increase in the knowledge of the proteins involved in cellulose synthesis. Despite this, there is still much to learn about cellulose synthesis, especially about how it is regulated at both transcriptional and post-transcriptional levels (Taylor, 2008, New Phytologist 178 (2), 239-252).

Typical primary fiber cell walls in *G. hirsutum*, which are about 0.5 μM thick and contain 20-25% cellulose along with pectin, xyloglucan, and protein (Meinert and Delmer 1977, Plant Physiol 59:1088-1097), are synthesized during fiber elongation (Haigler, 2007, In: R. M. Brown, Jr. and I. M. Saxena (eds.), *Cellulose: Molecular and Structural Biology*, 147-168, Springer). Primary wall deposition proceeds alone until 14-17 DPA, then a transition phase with concurrent primary and secondary wall deposition occurs between 15-24 DPA (representing deposition of the "winding layer"), followed by predominantly secondary wall synthesis until at least 40 DPA. The first period of wall thickening (12-16 DPA) is accomplished by continued synthesis in the same proportions of primary wall components (Meinert and Delmer, 1977, supra), an observation that is consistent with increasing wall birefringence while the cellulose microfibrils remain transversely oriented (Seagull, 1986, Can J Bot 64:1373-1381). The secondary wall finally attains a thickness of 3-6 μM around the whole circumference of the fiber, becoming thinner only at the fiber tip. In *G. barbadense*, there is an overlap between primary and secondary wall deposition within each fiber rather than in the fiber population because the overlapping period is greatly prolonged, and 90% of secondary wall deposition is complete before elongation ceases (DeLanghe, 1986, In: Mauney J. R. and Stewart J. McD. (eds.) cotton Physiology. The Cotton Foundation, Memphis, Tenn., pp. 325-350). It is thought that elongation continues exclusively at the fiber tip as secondary wall is deposited over most of the cell surface.

Maltby et al. (1979, Plant Physiol. 63, 1158-1164) describe that developing fibers of *Gossypium hirsutum* transiently synthesize 1,3-beta-D-glucan (callose) at the onset of secondary wall deposition followed by massive synthesis of cellulose. Meier et al. (1981, Nature 289: 821-822) describe that callose may be a probable intermediate in biosynthesis of cellulose of cotton fibers. DeLanghe (1986, supra) describes that callose may be required in cotton fiber secondary walls to provide a space for the crystallization and final orientation of cellulose microfibrils in the exoplasmic zone in the absence of typical matrix molecules.

The inventions described hereinafter in the different embodiments, examples, figures and claims provide improved methods and means for modulating fiber strength. More specifically, the present invention describes how to increase fiber strength and at the same time maintain a high fiber yield in plants. In particular, the invention describes how to increase fiber strength in cotton species selected for high yield, such as *Gossypium hirsutum*, by introgression of fiber strength determining genes from other cotton species selected for high fiber strength, such as *Gossypium barbadense*. Methods are also provided to identify molecular markers associated with fiber strength in a population of cotton varieties and related progenitor plants. The inventions described hereinafter also provide novel nucleic acid molecules encoding fiber-specific *Gossypium* glucanase proteins (GLUC1.1) and the proteins as such.

## SUMMARY OF THE INVENTION

The inventors identified a quantitative trait locus for fiber strength on chromosome A05 of *Gossypium* and found that *Gossypium barbadense* comprises an allele of this fiber strength locus that is superior to the allele of this QTL from *Gossypium hirsutum*, i.e. the presence of the *Gossypium barbadense* fiber strength allele in a *Gossypium* plant results in a higher fiber strength as compared to the fiber strength of a *Gossypium* plant comprising the *Gossypium hirsutum* fiber strength allele.

Thus, in a first aspect, the present invention provides a non-naturally occurring *Gossypium* plant, and parts and progeny thereof, comprising at least one superior allele of a fiber strength locus on chromosome A05.

In one embodiment, the plant is a plant from an A genome diploid *Gossypium* species, such as *Gossypium herbaceum* or *Gossypium arboreum*, or an AD genome allotetraploid *Gossypium* species, such as *Gossypium hirsutum* and *Gossypium barbadense*, and the superior fiber strength allele is derived from a different A or AD genome *Gossypium* species.

In another embodiment, the plant is a *Gossypium hirsutum*, a *Gossypium herbaceum* or a *Gossypium arboreum* plant, preferably a *Gossypium hirsutum* plant, and the superior fiber strength allele is derived from *Gossypium barbadense*.

In one aspect, the *Gossypium barbadense* fiber strength allele is located on chromosome A05 of *Gossypium barbadense* between AFLP marker P5M50-M126.7 and SSR marker CIR280. In another aspect, between AFLP marker P5M50-M126.7 and SSR marker BNL3992. In still another aspect, between AFLP marker P5M50-M126.7 and SSR marker CIR401c. In yet another aspect, is the LOD peak of the *Gossypium barbadense* fiber strength allele located between SSR marker NAU861 or the GLUC1.1 gene and SSR marker CIR401c. In a further aspect, is the LOD peak of the *Gossypium barbadense* fiber strength allele located at about 0 to 5 cM, more specifically at about 4.008 cM, from SSR marker NAU861 or the GLUC1.1 gene. In still a further aspect, is the LOD peak of the *Gossypium barbadense* fiber strength allele is located at about 0 to 12 cM, more specifically at about 10 cM, especially at about 10.52 cM, from SSR marker CIR401c.

In another aspect, the *Gossypium barbadense* fiber strength allele comprises at least one *Gossypium barbadense* ortholog of a nucleotide sequence comprised in the genomic DNA sequence spanning the *Gossypium hirsutum* GLUC1.1A gene represented in SEQ ID NO: 53.

In still another aspect, the *Gossypium barbadense* fiber strength allele comprises a GLUC1.1 gene encoding a non-functional GLUC1.1 protein. In one aspect, the *Gossypium barbadense* GLUC1.1 gene is characterised by the presence of a T nucleotide at a nucleotide position corresponding to nucleotide position 712 of SEQ ID NO: 5. In a further aspect, the *Gossypium barbadense* GLUC1.1 gene is located at about 0 to 5 cM, more specifically at about 4 cM, from the LOD peak of the *Gossypium barbadense* fiber strength allele. In yet a further aspect, the *Gossypium barbadense* GLUC1.1 gene is located at about 0 to 2 cM, at about 0 to 1 cM, more specifically at about 0.008 cM of the NAU861 marker.

In yet another embodiment, the plant is a *Gossypium hirsutum, Gossypium barbadense*, a *Gossypium herbaceum* or a *Gossypium arboreum* plant, preferably a *Gossypium hirsutum* plant, and the superior fiber strength allele is derived from *Gossypium* darwinii. In one aspect, the *Gossypium darwinii* fiber strength allele comprises a GLUC1.1 gene encoding a non-functional GLUC1.1 protein. In another aspect, the *Gossypium darwinii* GLUC1.1 gene is characterised by the presence of a T nucleotide at a nucleotide position corresponding to nucleotide position 761 of SEQ ID NO: 56.

In still another embodiment, the plant is a *Gossypium hirsutum, Gossypium barbadense* or a *Gossypium herbaceum* plant, preferably a *Gossypium hirsutum* plant, and the superior fiber strength allele is derived from *Gossypium arboreum*. In one aspect, the *Gossypium arboreum* fiber strength allele comprises a GLUC1.1 gene encoding a non-functional GLUC1.1 protein. In another aspect, the *Gossypium arboreum* GLUC1.1 gene is characterised by the absence of a C nucleotide at a nucleotide position corresponding to the nucleotide position between position 327 and 328 of SEQ ID NO: 21.

In a further embodiment, the callose content of the fibers is increased in the plant compared to the callose content of the fibers of an equivalent *Gossypium* plant that does not comprise the at least one superior allele of the fiber strength locus.

In yet a further embodiment, the strength of the fibers is increased in the plant compared to the strength of the fibers of an equivalent *Gossypium* plant that does not comprise the at least one superior allele of the fiber strength locus. In one aspect, the strength of the fibers is on average between about 5% and about 10%, preferably about 7.5%, higher. In another aspect, the strength of the fibers is on average between about 1.6 g/tex and about 3.3 g/tex, preferably about 2.5 g/tex, higher. In still another aspect, the strength of the fibers is on average between about 34.6 g/tex and about 36.3 g/tex, preferably about 35.5 g/tex.

In another embodiment, the plant is a *Gossypium hirsutum* plant homozygous for the *Gossypium barbadense* fiber strength allele.

In still another embodiment, the invention provides a fiber obtainable from the plant of any one of paragraphs 13 to 23.

In a further embodiment, the invention provides a method of identifying a *Gossypium barbadense* allele of a fiber strength locus on chromosome A05 in a plant, preferably a *Gossypium* plant, such as a *Gossypium hirsitum* plant, comprising the step of determining the presence of a *Gossypium barbadense* allele of a marker linked to the fiber strength locus in the genomic DNA of the plant selected from the group consisting of: AFLP marker P5M50-M126.7, SSR marker CIR280, SSR marker BNL3992, SSR marker CIR401c, SSR marker NAU861, a polymorphic site in an ortholog of a nucleotide sequence comprised in the genomic DNA sequence spanning a *Gossypium hirsutum* GLUC1.1A gene represented in SEQ ID NO: 53 of the plant; and a polymorphic site in a nucleotide sequence of a GLUC1.1A gene of the plant, such as SNP marker GLUC1.1A-SNP2 located at a nucleotide position corresponding to nucleotide position 418 to 428 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP3 located at a nucleotide position corresponding to nucleotide position 573 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP5 located at a nucleotide position corresponding to nucleotide position 712 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP6 located at a nucleotide position corresponding to nucleotide position 864 in SEQ ID NO: 5 or SNP marker GLUC1.1A-SNP8 located at a nucleotide position corresponding to nucleotide position 832 in SEQ ID NO: 5.

In a particular aspect, the *Gossypium barbadense* allele of AFLP marker P5M50-M126.7 is detected by amplification of a DNA fragment of about 126.7 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 43 and 44, respectively; the *Gossypium barbadense* allele of SSR marker CIR280 is detected by amplification of a DNA fragment of about 205 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 51 and 52, respectively; the *Gossypium barbadense* allele of SSR marker BNL3992 is detected by amplification of a DNA fragment of about 140 bp to about 145 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 49 and 50, respectively; the *Gossypium barbadense* allele of SSR marker CIR401c is detected by amplification of a DNA fragment of about 245 to about 250 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 47 and 48, respectively; the *Gossypium barbadense* allele of SSR marker NAU861 is detected by amplification of a DNA fragment of about 215 bp to about 220 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 45 and 46, respectively; the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP2 is detected by detecting a CTCATCAAA nucleotide sequence at a position corresponding to the position of SNP marker GLUC1.1A-SNP2 or by amplification of a DNA fragment of about 143 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 37 and 38, respectively; the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP3 is detected by detecting a C nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP3; the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP5 is detected by detecting a T nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP5; the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP6 is detected by detecting an A nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP6; the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP8 is detected by detecting a C nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP8.

In a further embodiment, the invention provides a method of identifying a *Gossypium darwinii* allele of a fiber strength locus on chromosome A05 in a plant, preferably a *Gossypium* plant, such as a *Gossypium hirsitum* plant, comprising the step of determining the presence of a *Gossypium darwinii* specific polymorphic site in a nucleotide sequence of a GLUC1.1A gene in the genomic DNA of the plant corresponding to the nucleotide sequence of a GLUC1.1A gene of SEQ ID NO: 56, such as SNP marker GLUC1.1A-SNP2 located at a nucleotide position corresponding to nucleotide position 476 to 477 in SEQ ID NO: 56, SNP marker GLUC1.1A-SNP3 located at a nucleotide position corresponding to nucleotide position 622 in SEQ ID NO: 56, SNP marker GLUC1.1A-SNP5 located at a nucleotide position corresponding to nucleotide position 761 in SEQ ID NO: 56, SNP marker GLUC1.1A-SNP6 located at a nucleotide position corresponding to nucleotide position 913 in SEQ ID NO: 56 or SNP marker GLUC1.1A-SNP8 located at a nucleotide position corresponding to nucleotide position 881 in SEQ ID NO: 56.

In a particular aspect, the *Gossypium darwinii* allele of SNP marker GLUC1.1A-SNP2 is detected by detecting a CTCATCAAA nucleotide sequence at a position corresponding to the position of SNP marker GLUC1.1A-SNP2 or by amplification of a DNA fragment of about 143 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 37 and 38, respectively; the *Gossypium darwinii* allele of SNP marker GLUC1.1A-SNP3 is detected by detecting a C nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP3; the *Gossypium darwinii* allele of SNP marker GLUC1.1A-SNP5 is detected by detecting a T nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP5; the *Gossypium darwinii* allele of SNP marker GLUC1.1A-SNP6 is detected by detecting an A nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP6, and the *Gossypium darwinii* allele of SNP marker GLUC1.1A-SNP8 is detected by detecting a G nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP8.

In a further embodiment, the invention provides a method of identifying a *Gossypium arboreum* allele of a fiber strength locus on chromosome A05 in a plant, preferably a *Gossypium* plant, such as a *Gossypium hirsitum* plant, comprising the step of determining the presence of a *Gossypium arboreum* specific polymorphic site in a nucleotide sequence of a GLUC1.1A gene in the genomic DNA of the plant corresponding to the nucleotide sequence of a GLUC1.1A gene of SEQ ID NO: 21, such as SNP marker GLUC1.1A-SNP7 located at a nucleotide position corresponding to a nucleotide position between nucleotide position 327 and 328 in SEQ ID NO: 21. In a particular aspect, the *Gossypium arboreum* allele of SNP marker GLUC1.1A-SNP7 is detected by detecting the absence of a C nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP7.

In a further embodiment, the invention provides a method of distinguishing a *Gossypium barbadense* allele of a fiber strength locus on chromosome A05 from a *Gossypium hirsutum* allele of the fiber strength locus in a plant, preferably a *Gossypium* plant, such as a *Gossypium hirsitum* plant, comprising the step of determining the presence of *Gossypium barbadense* alleles and/or *Gossypium hirsutum* alleles of markers linked to the fiber strength locus in the genomic DNA of the plant selected from the group consisting of: AFLP marker P5M50-M126.7, SSR marker CIR280, SSR marker BNL3992, SSR marker CIR401, SSR marker NAU861; a polymorphic site in an ortholog of a nucleotide sequence comprised in the genomic DNA sequence spanning the *Gossypium hirsutum* GLUC1.1A gene represented in SEQ ID NO: 53 of the plant; and a polymorphic site in a nucleotide sequence of a GLUC1.1A gene in the genomic DNA of the plant, such as SNP marker GLUC1.1A-SNP2 located at a nucleotide position corresponding to nucleotide position 418 to 428 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP3 located at a nucleotide position corresponding to nucleotide position 573 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP5 located at a nucleotide position corresponding to nucleotide position 712 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP6 located at a nucleotide position corresponding to nucleotide position 864 in SEQ ID NO: 5 or SNP marker GLUC1.1A-SNP8 located at a nucleotide position corresponding to nucleotide position 832 in SEQ ID NO: 5.

In a particular aspect, the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of AFLP marker P5M50-M126.7 bp amplification of, respectively, no DNA fragment and a DNA fragment of about 126.7 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 43 and 44, respectively; the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SSR marker CIR280 by amplification of, respectively, no DNA fragment and a DNA fragment of about 205 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 51 and 52, respectively; the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SSR marker BNL3992 by amplification of, respectively, two DNA fragments, one of about 160 bp to about 165 bp and one of about 85 bp to about 90 bp, and a DNA fragment of about 140 bp to about 145 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 49 and 50, respectively; the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SSR marker CIR401 bp amplification of, respectively, a DNA fragment of about 255 bp (CIR401b) and a DNA fragment of about 245 bp to about 250 bp (CIR401c) with at least two primers comprising at their extreme 3' end SEQ ID NO: 47 and 48, respectively; the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SSR marker NAU861 by amplification of, respectively, a DNA fragment of about 205 bp to about 210 bp and a DNA fragment of about 215 bp to about 220 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 45 and 46, respectively; the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP2 by detecting, respectively, no nucleotide or a CTCATCAAA nucleotide sequence at a position corresponding to the position of SNP marker GLUC1.1A-SNP2, or by amplification of, respectively, a DNA fragment of about 134 bp and a DNA fragment of about 143 bp with at least two primers comprising at their extreme 3' end SEQ ID NO: 37 and 38, respectively; the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP3 by detecting, respectively, a G or a C nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP3; the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP5 by detecting, respectively, a C or a T nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP5; the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP6 by detecting, respectively, a G or an A nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP6; and the *Gossypium hirsutum* allele is distinguished from the *Gossypium barbadense* allele of SNP marker GLUC1.1A-SNP8 by detecting, respectively, a G or a C nucleotide at a position corresponding to the position of SNP marker GLUC1.1A-SNP8.

In another embodiment, the invention provides a method for generating and/or selecting a non-naturally occurring *Gossypium* plant, and parts and progeny thereof, comprising at least one superior allele of a fiber strength locus on chromosome A05, wherein the superior fiber strength allele is derived from *Gossypium barbadense*, comprising the steps of crossing a plant from an A genome diploid *Gossypium* species, such as *Gossypium herbaceum* or *Gossypium arboreum*, or an AD genome allotetraploid *Gossypium* species, such as *Gossypium hirsutum*, with a *Gossypium barbadense* plant, and identifying the *Gossypium barbadense* fiber strength allele according to paragraph 25 or 26.

In another embodiment, the invention provides a method for generating and/or selecting a non-naturally occurring *Gossypium* plant, and parts and progeny thereof, comprising at least one superior allele of a fiber strength locus on chromosome A05, wherein the superior fiber strength allele is derived from *Gossypium* darwinii, comprising the steps of crossing a plant from an A genome diploid *Gossypium* species, such as *Gossypium herbaceum* or *Gossypium arboreum*, or an AD genome allotetraploid *Gossypium* species, such as *Gossypium hirsutum* or *Gossypium barbadense*, with a *Gossypium darwinii* plant, and identifying the *Gossypium darwinii* fiber strength allele according to paragraph 27 or 28.

In another embodiment, the invention provides a method for generating and/or selecting a non-naturally occurring *Gossypium* plant, and parts and progeny thereof, comprising at least one superior allele of a fiber strength locus on chromosome A05, wherein the superior fiber strength allele is derived from *Gossypium arboreum*, comprising the steps of crossing a plant from an A genome diploid *Gossypium* species, such as *Gossypium herbaceum*, or an AD genome allotetraploid *Gossypium* species, such as *Gossypium hirsutum* or *Gossypium barbadense*, with a *Gossypium arboreum* plant, and identifying the *Gossypium arboreum* fiber strength allele according to paragraph 29.

In still another embodiment, the invention provides a method for altering the callose content of a fiber in a *Gossypium* plant, particularly increasing the callose content of a fiber, comprising the steps of: introgressing a superior allele of the fiber strength locus on chromosome A05 in the *Gossypium* plant according to any one of paragraph 32 to 34, and selecting a plant with an altered callose content in its fibers, in particular an increased callose content.

In yet another embodiment, the invention provides a method for altering the properties of a fiber in a *Gossypium* plant, particularly increasing the strength of a fiber, comprising the steps of: introgressing a superior allele of the fiber strength locus on chromosome A05 in the *Gossypium* plant according to any one of paragraph 32 to 34, selecting a plant with an altered fiber strength, in particular an increased fiber strength.

In a further embodiment, the invention provides a kit for of identifying a *Gossypium barbadense* allele of a fiber strength locus on chromosome A05 or for distinguishing a *Gossypium barbadense* allele of a fiber strength locus on chromosome A05 from a *Gossypium hirsutum* allele of the fiber strength locus in a plant, preferably a *Gossypium* plant, such as a *Gossypium hirsitum* plant, comprising primers and/or probes for determining the presence of *Gossypium barbadense* alleles and/or *Gossypium hirsutum* alleles of markers linked to the fiber strength locus in the genomic DNA of the plant selected from the group consisting of: AFLP marker P5M50-M126.7, SSR marker CIR280, SSR marker BNL3992, SSR marker CIR401, SSR marker NAU861, a polymorphic site in an ortholog of a nucleotide sequence comprised in the genomic DNA sequence spanning the *Gossypium hirsutum* GLUC1.1A gene represented in SEQ ID NO: 53, and a polymorphic site in a nucleotide sequence of the GLUC1.1A gene in the genomic DNA of the plant, such as SNP marker GLUC1.1A-SNP2 located at a nucleotide position corresponding to nucleotide position 418 to 428 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP3 located at a nucleotide position corresponding to nucleotide position 573 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP5 located at a nucleotide position corresponding to nucleotide position 712 in SEQ ID NO: 5, SNP marker GLUC1.1A-SNP6 located at a nucleotide position corresponding to nucleotide position 864 in SEQ ID NO: 5 or SNP marker GLUC1.1A-SNP8 located at a nucleotide position corresponding to nucleotide position 832 in SEQ ID NO: 5.

In one aspect, the kit comprises at least two primers and/or probes selected from the group consisting of: primers comprising at their extreme 3' end SEQ ID NO: 43 and 44, respectively; primers comprising at their extreme 3' end SEQ ID NO: 51 and 52, respectively; primers comprising at their extreme 3' end SEQ ID NO: 49 and 50, respectively; primers comprising at their extreme 3' end SEQ ID NO: 47 and 48, respectively; primers comprising at their extreme 3' end SEQ ID NO: 45 and 46, respectively; primers comprising at their extreme 3' end SEQ ID NO: 37 and 38, respectively.

The inventors have further found that the properties of fibers in cotton plants can be controlled by controlling the number of endo-1,3-beta-glucanase genes/alleles that are "functionally expressed", i.e. that result in functional (biologically active) endo-1,3-beta-glucanase protein (GLUC), in fibers during the secondary cell wall synthesis phase and the maturation phase, herein commonly referred to as fiber strength building phase, of fiber development. By abolishing the functional expression of a number of endo-1,3-beta-glucanase genes/alleles that are functionally expressed in fibers during the fiber strength building phase, in particular during the maturation phase, of fiber development, such as the A-subgenome specific endo-1,3-beta-glucanase gene in *G. hirsutum*, while maintaining the functional expression of a number of such endo-1,3-beta-glucanase genes/alleles, such as the D-subgenome specific endo-1,3-beta-glucanase gene in *G. hirsutum*, it is believed that the degradation of callose can be decreased to a level allowing a higher fiber strength, while maintaining a level of callose degradation sufficient to obtain an industrially relevant fiber length.

Thus, in another aspect, the present invention provides a non-naturally occurring fiber-producing plant, and parts and progeny thereof, characterized in that the functional expression of at least one allele of at least one fiber-specific GLUC gene that is functionally expressed during the fiber strength building phase, in particular the fiber maturation phase, of fiber development is abolished. Such plants, and parts and progeny thereof, can be used for obtaining plants with modified callose content and/or modified fiber properties, in particular for obtaining fiber-producing plants with increased callose content in the fibers and/or increased fiber strength that preferably maintain an industrially relevant fiber length. As used herein, "plant part" includes anything derived from a plant of the invention, including plant parts such as cells, tissues, organs, seeds, fibers, seed fats or oils.

In one embodiment, the GLUC gene is a GLUC1.1 gene encoding a GLUC protein that has at least 90% sequence identity to SEQ ID NO: 4.

In another embodiment, the plant is a *Gossypium* plant, wherein the GLUC gene is a GLUC1.1A gene encoding a GLUC protein that has at least 97% sequence identity to SEQ ID NO: 4 or a GLUC1.1D gene encoding a GLUC protein that has at least 97% sequence identity to SEQ ID NO: 10, preferably the GLUC1.1A gene.

In still another embodiment, the plant is a *Gossypium hirsutum* plant.

In a further embodiment, the amount of functional GLUC protein is significantly reduced in fibers during the fiber strength building phase, in particular the fiber maturation phase, of fiber development in the plant compared to the amount of functional GLUC protein produced in fibers during the fiber strength building phase, in particular the fiber maturation phase, of fiber development in a plant in which the functional expression of the at least one GLUC allele is not abolished.

In still a further embodiment, the callose content is significantly increased in fibers of the plant compared to the callose content in fibers in a plant in which the functional expression of the at least one GLUC allele is not abolished.

In yet a further embodiment, the strength of the fibers is significantly increased compared to the strength of the fibers in a plant in which the functional expression of the at least one GLUC allele is not abolished. In one aspect, the strength of the fibers is on average between about 5% and about 10%, preferably about 7.5%, higher. In another aspect, the strength of the fibers is on average between about 1.6 g/tex and about 3.3 g/tex, preferably about 2.5 g/tex, higher. In still another aspect, the strength of the fibers is on average between about 34.6 g/tex and about 36.3 g/tex.

In still a further embodiment, the plant is a *Gossypium hirsutum* plant characterized in that the functional expression of at least two alleles of at least one fiber-specific GLUC gene is abolished.

In another embodiment, the present invention provides a fiber obtainable from the fiber-producing plant of any one of paragraphs 40 to 47.

In a further embodiment, the present invention provides a nucleic acid molecule encoding a non-functional GLUC1.1 protein having an amino acid sequence wherein at least one amino acid residue similar to the active site residues or to the glycosylation site residues of the GLUC1.1 protein of SEQ ID NO: 4 is lacking or is substituted for a non-similar amino acid residue. In one aspect, the active site residues of the GLUC1.1 protein of SEQ ID NO: 4 are selected from the group consisting of Tyr48, Glu249, Trp252, and Glu308, and wherein the glycosylation site residue of the GLUC1.1 protein of SEQ ID NO: 4 is Asn202. In another aspect, the non-functional GLUC1.1 protein comprises an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO: 6, SEQ ID NO: 18, SEQ ID NO: 57 or SEQ ID NO: 22. In another aspect, the nucleic acid molecule comprises a nucleic acid sequence having at least 92% sequence identity to SEQ ID NO: 3 from nucleotide 101 to 1078, wherein at least one nucleic acid residue is deleted, inserted or substituted. In yet another aspect, the nucleic acid molecule comprises a nucleotide sequence at least 92% identical to the nucleic acid sequence of SEQ ID NO: 54 from nucleotide 50 to 589. In still a further aspect, the nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO: 54 from nucleotide 50 to 589. In still another aspect, the nucleic acid molecule comprises a nucleic acid sequence having at least 92% sequence identity to SEQ ID NO: 1 from nucleotide 2410 to 3499, wherein at least one nucleic acid residue is deleted, inserted or substituted. In yet another aspect, the nucleic acid molecule comprises a nucleotide sequence at least 92% identical to the nucleic acid sequence of SEQ ID NO: 5 from nucleotide 63 to 711, SEQ ID NO: 17 from nucleotide 2 to 472, SEQ ID NO: 56 from nucleotide 112 to 760 or SEQ ID NO: 21 from nucleotide 27 to 372. In still a further aspect, the nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO: 5 from nucleotide 63 to 711, SEQ ID NO: 17 from nucleotide 2 to 472, SEQ ID NO: 56 from nucleotide 112 to 760, or SEQ ID NO: 21 from nucleotide 27 to 372.

In another embodiment, the present invention provides a non-functional GLUC1.1 protein encoded by the nucleic acid molecule of paragraph 49.

In still another embodiment, the present invention provides a method for identifying a GLUC1.1 gene encoding a non-functional GLUC1.1 protein in a plant, preferably a *Gossypium* plant, such as a *Gossypium hirsitum* plant, said GLUC1.1 gene comprising a nucleic acid sequence having at least 92% sequence identity to SEQ ID NO: 1 from nucleotide 2410 to 3499, comprising the step of identifying a polymorphic site in the nucleotide sequence of the GLUC1.1 gene in the genomic DNA of the plant that results in the production of a non-functional GLUC1.1 protein. In one aspect, the present invention provides a method for identifying a GLUC1.1 gene from *Gossypium barbadense* or from *Gossypium darwinii* comprising the step of identifying a T nucleotide at a nucleotide position corresponding to nucleotide position 3050 in SEQ ID NO: 1. In another aspect, the present invention provides a method for identifying a GLUC1.1 gene from *Gossypium arboreum* comprising the step of identifying a deletion of a C nucleotide at a nucleotide position corresponding to nucleotide position 2674, 2675 or 2676 in SEQ ID NO: 1.

In a further embodiment, the present invention provides a method of distinguishing a GLUC1.1 gene encoding a non-functional GLUC1.1 protein from a GLUC1.1 gene encoding a functional GLUC1.1 protein, said GLUC1.1 genes both comprising a nucleic acid sequence having at least 92% sequence identity to SEQ ID NO: 1 from nucleotide 2410 to 3499, comprising the step of identifying a polymorphic site in the nucleotide sequences of the GLUC1.1 genes. In one aspect, the present invention provides a method of distinguishing a GLUC1.1 from *Gossypium barbadense*, from *Gossypium darwinii* or from *Gossypium arboreum* from a GLUC1.1 gene from *Gossypium hirsutum*, respectively, comprising the step of identifying a polymorphic site selected from the group consisting of: polymorphic sequence marker GLUC1.1A-SNP2 located between the nucleotide at position 2765 and 2766 in SEQ ID NO: 1, SNP marker GLUC1.1A-SNP3 located at nucleotide position 2911 in SEQ ID NO: 1, SNP marker GLUC1.1A-SNP5 located at nucleotide position 3050 in SEQ ID NO: 1, SNP marker GLUC1.1A-SNP6 located at nucleotide position 3202 in SEQ ID NO: 1, SNP marker GLUC1.1A-SNP7 located at nucleotide position 2674, 2675 or 2676 in SEQ ID NO: 1 and SNP marker GLUC1.1A-SNP8 located at nucleotide position 3170 in SEQ ID NO: 1. In another aspect, polymorphic sequence marker GLUC1.1A-SNP2 from *Gossypium barbadense* or *Gossypium darwinii* and from *Gossypium hirsutum*, respectively, is detected by amplification of a DNA fragment of about 143 bp and about 134 bp, respectively, with primers comprising at their extreme 3' end SEQ ID NO: 37 and 38, respectively. In still another aspect, SNP marker GLUC1.1A-SNP3 from *Gossypium barbadense* or *Gossypium darwinii* and from *Gossypium hirsutum*, respectively, is detected by amplification of a DNA fragment of about 57 bp with primers comprising SEQ ID NO: 41 and 42 and detection of the DNA fragment with fluorescently labeled probes comprising SEQ ID NO: 39 and 40, respectively.

In a further embodiment, the present invention provides a method for generating and/or selecting a non-naturally occurring fiber-producing plant, and parts and progeny thereof, wherein the functional expression of at least one allele of at least one fiber-specific GLUC gene that is functionally expressed during the fiber strength building phase, in particular the fiber maturation phase, of fiber development is abolished, comprising the step of: mutagenizing at least one allele of the GLUC gene, or introgressing at least one allele of a non-functionally expressed ortholog of the GLUC gene or at least one allele of a mutagenized GLUC gene, or introducing a chimeric gene comprises the following operably linked DNA elements: (a) a plant expressible promoter, (b) a transcribed DNA region, which when transcribed yields an inhibitory RNA molecule capable of reducing the expression of the GLUC allele, and (c) a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of the plant. In one aspect, the GLUC gene is a GLUC1.1 gene encoding a GLUC protein that has at least 90% sequence identity to SEQ ID NO: 4. In another aspect, the fiber-producing plant is a *Gossypium* plant, and the GLUC gene is a GLUC1.1A gene encoding a GLUC protein that has at least 97% sequence identity to SEQ ID NO: 4 or a GLUC1.1D gene encoding a GLUC protein that has at least 97% sequence identity to SEQ ID NO: 9, preferably a GLUC1.1A gene. In still another aspect, the fiber-producing plant is a *Gossypium* plant, and the non-functionally expressed ortholog of the GLUC gene is a GLUC1.1A gene which is derived from a *Gossypium barbadense*, from a *Gossypium darwinii* or a *Gossypium arboreum* plant, preferably from a *Gossypium barbadense*. In a further aspect, the method further comprises the step of identifying the non-functionally expressed ortholog of the GLUC gene or the mutagenized GLUC gene according to the method of paragraph 51.

In a further embodiment, the present invention provides a method for altering the callose content of a fiber in a fiber-producing plant, particularly increasing the callose content of a fiber, comprising the steps of: generating and/or selecting a non-naturally occurring fiber-producing plant, and parts and progeny thereof, wherein the functional expression of at least one allele of at least one fiber-specific GLUC gene that is functionally expressed during the fiber strength building phase, in particular the fiber maturation phase, of fiber development is abolished, according to the method of paragraph 53, and selecting a plant with an altered callose content in its fibers, in particular an increased callose content.

In a further embodiment, the present invention provides a method for altering the properties of a fiber in a fiber-producing plant, particularly increasing the strength of a fiber, comprising the steps of: generating and/or selecting a non-naturally occurring fiber-producing plant, and parts and progeny thereof, wherein the functional expression of at least one allele of at least one fiber-specific GLUC gene that is functionally expressed during the fiber strength building phase, in particular the fiber maturation phase, of fiber development is abolished, according to the method of paragraph 53, and selecting a plant with an altered fiber strength, in particular an increased fiber strength.

In another embodiment, the present invention provides a kit for identifying a GLUC1.1 gene encoding a non-functional GLUC1.1 protein in a plant, said GLUC1.1 gene comprising a nucleic acid sequence having at least 92% sequence identity to SEQ ID NO: 1 from nucleotide 2410 to 3499, comprising primers and/or probes for determining the presence of a polymorphic site in the nucleotide sequence of the GLUC1.1 gene in the genomic DNA of the plant that results in the production of a non-functional GLUC1.1 protein. In one aspect, the kit comprises primers and/or probes for determining the presence of a T nucleotide at a nucleotide position corresponding to nucleotide position 3050 in SEQ ID NO: 1 or for determining a deletion of a C nucleotide at a nucleotide position corresponding to nucleotide position 2674, 2675 or 2676 in SEQ ID NO: 1.

In still another embodiment, the present invention provides a kit for distinguishing a GLUC1.1 gene encoding a non-functional GLUC1.1 protein from a GLUC1.1 gene encoding a functional GLUC1.1 protein, said GLUC1.1 genes both comprising a nucleic acid sequence having at least 92% sequence identity to SEQ ID NO: 1 from nucleotide 2410 to 3499, comprising primers and/or probes for determining the presence of a polymorphic site in the nucleotide sequences of the GLUC1.1 genes. In one aspect, the present invention provides a kit comprising primers and/or probes for distinguishing *Gossypium barbadense, Gossypium* darwinii or *Gossypium arboreum* specific alleles from *Gossypium hirsutum* specific alleles of a polymorphic site selected from the group consisting of: polymorphic sequence marker GLUC1.1A-SNP2 located between the nucleotide at position 2765 and 2766 in SEQ ID NO: 1, SNP marker GLUC1.1A-SNP3 located at nucleotide position 2911 in SEQ ID NO: 1, SNP marker GLUC1.1A-SNP5 located at nucleotide position 3050 in SEQ ID NO: 1, SNP marker GLUC1.1A-SNP6 located at nucleotide position 3202 in SEQ ID NO: 1, SNP marker GLUC1.1A-SNP7 located at nucleotide position 2674, 2675 or 2676 in SEQ ID NO: 1 and SNP marker GLUC1.1A-SNP8 located at nucleotide position 3170 in SEQ ID NO: 1. In another aspect, the kit comprises at least two primers and/or probes selected from the group consisting of: primers comprising at their extreme 3' end SEQ ID NO: 37 and 38, respectively, to identify polymorphic sequence marker GLUC1.1A-SNP2, primers comprising SEQ ID NO: 41 and 42, respectively, to identify SNP marker GLUC1.1A-SNP3, probes comprising SEQ ID NO: 39 and 40, respectively, to identify SNP marker GLUC1.1A-SNP3, primers comprising SEQ ID NO: 62 and 63, respectively, to identify SNP marker GLUC1.1A-SNP5, and probes comprising SEQ ID NO: 60 and 61, respectively, to identify SNP marker GLUC1.1A-SNP5.

## BRIEF DESCRIPTION OF THE FIGURES

FIG. **1**: Alignment of genomic and cDNA sequences of A and D subgenome-specific GLUC1.1 genes from *Gossypium hirsutum* ('GhGLUC1.1A-gDNA' corresponds to SEQ ID NO: 1 from nucleotide 2246 to 3753, 'GhGLUC1.1A-cDNA' corresponds to SEQ ID NO: 3, 'GhGLUC1.1D-gDNA' corresponds to SEQ ID NO: 7 from nucleotide 3206 to 4694, and 'GhGLUC1.1D-cDNA' corresponds to SEQ ID NO: 9) and *Gossypium barbadense* ('GbGLUC1.1A-gDNA' corresponds to SEQ ID NO: 5, 'GbGLUC1.1A-cDNA' corresponds to SEQ ID NO: 54, 'GbGLUC1.1D-gDNA' corresponds to SEQ ID NO: 11, and 'GbGLUC1.1D-cDNA' corresponds to SEQ ID NO: 13). The putative TATA box is indicated in bold, the putative start codons and the putative first exons are indicated in bold and in bold with an arrow, respectively, the putative intron and second exon sequences are indicated in regular with an arrow, the putative intron sequences are further indicated between '/', the putative (premature) STOP codons are indicated in italic and underlined.

FIG. **2**: Alignment of amino acid sequences of A and D subgenome-specific GLUC1.1 proteins from *Gossypium hirsutum* ('GhGLUC1.1A' corresponds to SEQ ID NO: 2 and 4 and 'GhGLUC1.1D' corresponds to SEQ ID NO: 8 and 10) and *Gossypium barbadense* ('GbGLUC1.1A' corresponds to SEQ ID NO: 6 and 55 and 'GbGLUC1.1D' corresponds to SEQ ID NO: 12 and 14). The putative signal peptide is indicated in italic, the putative post-translational splicing site is indicated as '><', the GH17 signature is indicated in bold. Amino acids that are identical between at least three of the four sequences are highlighted. The dashed line indicates the protein segment that is missing in GbGLUC1.1A.

FIG. **3**: Protein model of GLUC1.1A protein of *G. hirsutum* (FIG. **3***a*; right) and *G. barbadense* (FIG. **3***b*; right) based on an X-ray structure of a barley 1,3-1,4-beta-glucanase (laq0; FIG. **3***a* &*b*; left). The active site of laq0 is located in an open cleft at the bottom of the barrel defined by the C-terminal ends of the parallel intra-barrel beta-strands (Müller et al., 1998, J. Biol. Chem. 273 (6): 3438-3446) and is indicated by the amino acids and their position numbers displayed in the upper left part of the protein model of 1aq0 in FIG. **3***a* and *b* at the left. Active site residues Glu288, Glu232 and Tyr33 in 1aq0 (FIG. **3***a*, left) correspond to Glu308, Glu249 and Tyr48 in GhGLUC1.1A (FIG. **3***a*, right) and are absent in GbGLUC1.1A (FIG. **3***b*, right). The glycosylation site Asn190 in 1aq0 (FIG. **3**A, left) corresponds to Asn 202 in GhGLUC1.1A (FIG. **3***a*, right) and is also absent in GbGLUC1.1A (FIG. **3***b*, right). FIG. **3***b* further shows that the threonine, histidine and glutamine amino acids at position 82, 83 and 84 of GbGLUC1.1A (FIG. **3***b*; right) that are not present in GhGLUC1.1A (see for example FIG. **7**) are located in a distant loop which is not part of the active site and not involved in glycosylation.

FIG. **4**: Box plot indicating the difference in fiber strength (as determined by measuring the breaking force of single fibers; indicated in cN on the Y-axis) between untreated fibers ('untreated') and fibers treated with exogenous glucanase ('treated') derived from *Gossypium hirsutum* cultivar FM966 grown in a greenhouse in Europe ('FM966 Astene'), in the field in the US ('FM966 Sellers') and in the field in Australia ('FM966 Australia'), from *Gossypium hirsutum* cultivar Coker 312 grown in a greenhouse in Europe ('Coker 312'), from *Gossypium barbadense* cultivar PimaS7 grown in a greenhouse in Europe ('PimaS7'), and from *Gossypium barbadense* cultivar PimaY5 grown in the field in Australia ('PimaY5').

FIG. **5**: Box plot indicating the difference in callose content (as determined by fluorescence measurements of aniline blue stained fibers; indicated as the ratio of green over blue fluorescence on the Y-axis) between untreated fibers ('untreated') and fibers treated with exogenous glucanase ('treated') derived from *Gossypium hirsutum* cultivar FM966 grown in a greenhouse in Europe ('FM966 Astene'), in the field in the US ('FM966 Sellers') and in the field in Australia ('FM966 Australia'), from *Gossypium hirsutum* cultivar Coker 312 grown in a greenhouse in Europe ('Coker 312'), from *Gossypium barbadense* cultivar PimaS7 grown in a greenhouse in Europe ('PimaS7'), and from *Gossypium barbadense* cultivar PimaY5 grown in the field in Australia ('PimaY5').

FIG. **6**: Alignment of genomic DNA sequences of A and D subgenome-specific GLUC1.1 genes from *Gossypium hirsutum* ('GhGLUC1.1A_gDNA' corresponds to SEQ ID NO: 1 from nucleotide 2348 to 3554 and 'GhGLUC1.1D_gDNA' corresponds to SEQ ID NO: 7 from nucleotide 3311 to 4496), *Gossypium tomentosum* ('GtGLUC1.1A_gDNA' corresponds to SEQ ID NO: 15 and 'GtGLUC1.1D_gDNA' corresponds to SEQ ID NO: 25), *Gossypium barbadense* ('GbGLUC1.1A_gDNA' corresponds to SEQ ID NO: 5 and 'GbGLUC1.1D_gDNA' corresponds to SEQ ID NO: 11), *Gossypium darwinii* ('GdGLUC1.1A_gDNA' corresponds to SEQ ID NO: 17 and 'GdGLUC1.1D_gDNA' corresponds to SEQ ID NO: 27), *Gossypium mustelinum*, ('GmGLUC1.1A_gDNA' corresponds to SEQ ID NO: 19 and 'GmGLUC1.1D_gDNA' corresponds to SEQ ID NO: 29), *Gossypium arboreum* ('GaGLUC1.1A_gDNA' corresponds to SEQ ID NO: 21), *Gossypium herbaceum* ('GheGLUC1.1A_gDNA' corresponds to SEQ ID NO: 23), and *Gossypium raimondii* ('GrGLUC1.1D_gDNA' corresponds to SEQ ID NO: 31). The positions of primers SE077 and SE078, used to generate the complete coding sequence from start to stop codon, and the positions of primers SE003 and SE002, used to generate partial coding sequences, are underlined. The putative start codons and the putative first exons are indicated in bold and in bold with an arrow, respectively, the putative intron and second exon sequences are indicated in regular with an arrow, the putative intron sequences are further indicated between '/', the putative (premature) STOP codons are indicated in italic and underlined. Five polymorphic sites (4 single nucleotide polymorphisms (SNPs) and one extended indel) that exist between the GLUC1.1A or GLUC1.1D sequences of, e.g., *G. hirsutum* FM966 and *G. barbadense* Pima S7 or *G. darwinii*, are indicated with arrows and named 'GLUC1.1D-SNP1' and 'GLUC1.1A-SNP2, 3, 5 and 6'. Allelic variants are indicated as follows: [*G. hirsutum* allele/*G. barbadense* or *G. darwinii* allele]. One polymorphic site (1 SNP) that exist between the GLUC1.1A sequences of, e.g., *G. hirsutum* FM966 and *G. arboreum* is indicated with an arrow and named 'GLUC1.1A-SNP7'. Allelic variants are indicated as follows: [*G. hirsutum* allele/*G. arboreum* allele]. One polymorphic site (1 SNP) that exist between the GLUC1.1A sequences of, e.g., *G. barbadense* Pima S7 or *G. darwinii* is indicated with an arrow and named 'GLUC1.1A-SNP8'. Allelic variants are indicated as follows: [*G. barbadense* allele/*G. darwinii* allele].

FIG. **7**: Alignment of amino acid sequences of A and D subgenome-specific GLUC1.1 proteins from *Gossypium hirsutum* (GhGLUC1.1A_prot' corresponds to SEQ ID NO: 2 and 4 and GhGLUC1.1D__ prot' corresponds to SEQ ID NO: 8 and 10; full-length sequences), *Gossypium tomentosum* (GtGLUC1.1A_prot' corresponds to SEQ ID NO: 16 and GtGLUC1.1D_prot' corresponds to SEQ ID NO: 26; partial sequences), *Gossypium barbadense* (GbGLUC1.1A_prot' corresponds to SEQ ID NO: 6 and 55 and GbGLUC1.1D_prot' corresponds to SEQ ID NO: 12 and 14; full-length sequences), *Gossypium darwinii* (GdGLUC1.1A_prot' corresponds to SEQ ID NO: 57 and GdGLUC1.1D_prot' corresponds to SEQ ID NO: 59; full-length sequences), *Gossypium mustelinum*, (GmGLUC1.1A_prot' corresponds to SEQ ID NO: 20 and GmGLUC1.1D_prot' corresponds to SEQ ID NO: 30; partial sequences), *Gossypium arboreum* (GaGLUC1.1A_prot' corresponds to SEQ ID NO: 22; full-length sequence), *Gossypium herbaceum* (GheGLUC1.1A_proe corresponds to SEQ ID NO: 24; full-length sequence), and *Gossypium raimondii* (GrGLUC1.1D_prot' corresponds to SEQ ID NO: 32; partial sequences). The putative signal peptide is indicated in italic, the putative post-translational splicing site is indicated as '><', the GH17 signature is indicated in bold. Amino acids that differ from the amino acids in the upper sequence, i.e. GhGLUC1.1A_prot, are highlighted.

FIG. **8**: Expression of GLUC1.1A and GLUC1.1D in *G. barbadense*. DNA from a cDNA library from (developing) fibers in *Gossypium barbadense* was extracted and equalized. PCR fragments were amplified using oligonucleotide primers SE002 and SE003 (SEQ ID NO: 35 and 36) and digested with restriction enzyme AlwI. A PCR amplified product for GLUC1.1A yields 3 fragments (479 bp118 bp+59 bp) while for GLUC1.1D it only yields 2 fragments (538 bp+118 bp). The 59 bp fragment is not visible. Lane 1 and 12: 1 kb size markers; lanes 2 to 9: GbGLUC1.1A and D expression at 0, 5, 10, 15, 20, 25, 30 and 40 DPA; lane 10: negative (no template; NTC); lane 11: positive control (genomic DNA from Pima S7).

FIG. **9**: Schematic representation of 165250 bps DNA fragment spanning the GLUC1.1A gene of *Gossypium hirsutum* (SEQ ID NO: 53). Box: retrotransposon region; *: position of CIR280 homology region; arrow: DNA fragment encoding protein indicated with following abbreviations: SHMT (Serine HydroxyMethylTransferase); GrpE/HSP-70 (GrpE protein/HSP-70 cofactor); ARF17: putative Auxin Response Factor similar to At-ARF17; eIF-5-1: probable eukaryotic translation Initiation Ffactor 5-1; Avr9: putative Avr9 elicitor response protein; VPS9: similar to Vacuolar Protein Sorting-associated protein VPS9; HAT: putative Histon Acetyl Transferase gene; Gluc1.1: GLUC1.1A encoding region; MEKK1: putative Mitogen-activated protein kinase kinase kinase 1; PIP5K1: Phosphatidylinositol-4-Phosphate 5-Kinase 1.

## DETAILED EMBODIMENTS

The current invention is based on the unexpected finding that the presence of the *Gossypium barbadense* ortholog of a fiber strength locus on chromosome A05, hereinafter called *Gossypium barbadense* fiber strength allele, in *Gossypium hirsutum* plants results in an increased strength of the fibers of the *Gossypium hirsutum* plants compared to the strength of the fibers of *Gossypium hirsutum* plants comprising the *Gossypium hirsutum* ortholog of the fiber strength locus.

Thus, in a first aspect, the present invention provides a non-naturally occurring *Gossypium* plant, and parts and progeny thereof, comprising at least one superior allele of a quantitative trait locus (QTL) for fiber strength located on chromosome A05.

As used herein, the term "non-naturally occurring" or "cultivated" when used in reference to a plant, means a plant with a genome that has been modified by man. A transgenic fiber-producing plant, for example, is a non-naturally occurring fiber-producing plant that contains an exogenous nucleic acid molecule, e.g., a chimeric gene comprising a transcribed region which when transcribed yields a biologically active RNA molecule capable of reducing the expression of a GLUC gene according to the invention and, therefore, has been genetically modified by man. In addition, a fiber-producing plant that contains, for example, a mutation in an endogenous GLUC gene (e.g. in a regulatory element or in the coding sequence) as a result of an exposure to a mutagenic agent is also considered a non-naturally occurring fiber-producing plant, since it has been genetically modified by man. Furthermore, a fiber-producing plant of a particular species, such as *Gossypium hirsutum*, that contains, for example, a mutation in an endogenous GLUC gene that in nature does not occur in that particular plant species, as a result of, for example, directed breeding processes, such as marker-assisted breeding and selection or introgression, with another species of that fiber-producing plant, such as *Gossypium barbadense*, is also considered a non-naturally occurring fiber-producing plant. In contrast, a fiber-producing plant containing only spontaneous or naturally occurring mutations, i.e. a plant that has not been genetically modified by man, is not a "non-naturally occurring plant" as defined herein and, therefore, is not encompassed within the invention. One skilled in the art understands that, while a non-naturally occurring fiber-producing plant typically has a nucleotide sequence that is altered as compared to a naturally occurring fiber-producing plant, a non-naturally occurring fiber-producing plant also can be genetically modified by man without altering its nucleotide sequence, for example, by modifying its methylation pattern.

The term "quantitative trait" refers herein to a trait, such as fiber strength, whose phenotypic characteristics vary in degree and can be attributed to the interactions between two or more genes and their environment.

As used herein, the term "locus" (loci plural) or "site" means a specific place or places on a chromosome where, for example, a gene, a genetic marker or a QTL is found.

A "quantitative trait locus (QTL)" is a stretch of DNA (such as a chromosome arm, a chromosome region, a nucleotide sequence, a gene, and the like) that is closely linked to a gene that underlies the trait in question. "QTL mapping" involves the creation of a map of the genome using genetic or molecular markers, like AFLP, RAPD, RFLP, SNP, SSR, and the like, visible polymorphisms and allozymes, and determining the degree of association of a specific region on the genome to the inheritance of the trait of interest. As the markers do not necessarily involve genes, QTL mapping results involve the degree of association of a stretch of DNA with a trait rather than pointing directly at the gene responsible for that trait. Different statistical methods are used to ascertain whether the degree of association is significant or not. A molecular marker is said to be "linked" to a gene or locus, if the marker and the gene or locus have a greater association in inheritance than would be expected from independent assortment, i.e. the marker and the locus co-segregate in a segregating population and are located on the same chromosome. "Linkage" refers to the genetic distance of the marker to the locus or gene (or two loci or two markers to each other). The closer the linkage, the smaller the likelihood of a recombination event taking place, which separates the marker from the gene or locus. Genetic distance (map distance) is calculated from recombination frequencies and is expressed in centiMorgans (cM) [Kosambi (1944), *Ann. Eugenet.* 12:172-175].

"Fiber strength locus" or "strength locus", as used herein, refers to a stretch of DNA on chromosome A05 of *Gossypium* species that is closely linked to (a) gene(s) that is(are) involved in the regulation of fiber strength. The "fiber strength locus" is a QTL said to be linked to the "(fiber strength) causal gene(s)".

A "fiber", such as a "cotton fiber", as used herein, refers to a seed trichome, more specifically a single cell of a fiber-producing plant, such as cotton, that initiates from the epidermis of the outer integument of the ovules, at or just prior to anthesis. The morphological development of cotton fibers has been well documented (Basra and Malik, 1984, Int Rev of Cytology 89: 65-113; Graves and Stewart, 1988, supra; Ramsey and Berlin, 1976, American Journal of Botany 63 (6): 868-876; Ruan and Chourey, 1998, Plant Physiology 118: 399-406; Ruan et al. 2000, Aust. J. Plant Physiol. 27:795-800; Stewart, 1975, Am. J. Bot. 62, 723-730). Cotton fibers, in particular from *Gossypium hirsutum*, undergo four overlapping developmental stages: fiber cell initiation, elongation, secondary cell wall biosynthesis, and maturation. Fiber cell initiation is a rapid process. White fuzzy fibers begin to develop immediately after anthesis and continue up to about 3 days post-anthesis (DPA), which is followed by fiber cell elongation (until about 10 to about 17 DPA). Depending upon growth conditions, secondary cell wall biosynthesis initiates and continues to about 25 to about 40 DPA, followed by a maturation process until about 45 to about 60 DPA. The secondary cell wall synthesis and maturation phase are herein commonly referred to as "fiber strength building phase". Only about 25 to 30% of the epidermal cells differentiate into the commercially important lint fibers (Kim and Triplett, 2001). The majority of cells does not differentiate into fibers or develop into short fibers or fuzz. During fiber elongation and secondary wall metabolism, the fiber cells elongate rapidly, synthesize secondary wall components, and show dramatic cellular, molecular and physiological changes. Fiber elongation is coupled with rapid cell growth and expansion (Seagull, 1991. In *Biosynthesis and biodegradation of cellulose* (Haigler, C. H. & Weimer, P. J., eds) pp. 1432163, MarcelDekker, New York) and constant synthesis of a large amount of cell metabolites and cell wall components such as cellulose. About 95% of the dry-weight in mature cotton fibers is cellulose (Pfluger and Zambryski, 2001, Curr Biol 11: R436-R439; Ruan et al., 2001, Plant Cell 13: 47-63). Non-celluloid components are also important to fiber cell development (Hayashi and Delmer, 1988, Carbohydr. Res. 181: 273-277; Huwyler et al., 1979, Planta 146: 635-642; Meinert and Delmer, 1977, Plant Physiol 59: 1088-1097; Peng et al., 2002, Science 295: 147-150). Compared to other plant cells, cotton fibers do not contain lignin in secondary walls but have large vacuoles that are presumably related to rapid cell growth and expansion (Basra and Malik, 1984, supra; Kim and Triplett, 2001, Plant Physiology 127: 1361-1366; Mauney, 1984, supra; Ruan and Chourey, 1998, supra; Ruan et al., 2000, supra; Van't H of, 1999, American Journal of Botany 86: 776-779).

"Fiber strength", as used herein, can be determined by determining the strength of a bundle of fibers, i.e. "fiber bundle strength", or by determining the strength of single fibers. The higher the single fiber strength and the lower the variations of single fiber breaking elongation, the closer the bundle and yarn tensile strength would be to the sum of single fiber strength; ideally, fiber bundle tenacity would equal the total single fiber breaking tenacity had all fibers within the bundle equal breaking elongation and no slack (Liu et al., February 2005, Textile Res. J).

"Fiber bundle strength", as used herein, refers to a measure that is usually expressed in terms of grams per tex. This commercial High Volume Instruments (HVI) measure of fiber bundle strength ("HVI strength") is also called "tenacity". A tex unit is equal to the weight in grams of 1,000 meters of fiber. Therefore, the strength reported is the force in grams required to break a bundle of fibers one tex unit in size. Measurements of cotton fiber bundle strength can, for example, be made according to USDA standards. A beard of cotton is clamped in two sets of jaws, one eighth inch apart, and the force required to break the fibers is determined. Table 1 can be used as a guide in interpreting fiber strength measurements.

TABLE 1

Interpretation of HVI fiber strength measurements

| Degree of Strength | HVI* Strength (grams per tex) |
|---|---|
| Very Strong | 31 or more |
| Strong | 29-30 |
| Average | 26-28 |
| Intermediate | 24-25 |
| Weak | 23 or less |

*High Volume Precision Instruments

Alternatively, the strength of fibers can be compared by determining the "single fiber strength" by performing single fiber tensile tests, for example, on a FAVIMAT Robot (Textechno) as described on the World Wide Web at textechno-.com in the Examples. Briefly, a single fiber is clamped between two fiber clamps with a continuously adjustable gauge length between 5 and 100 mm (set e.g. on 8 mm) and a draw-off clamp speed between 0.1 and 100 mm/min (set e.g. on 4 mm/min), and the force (cN) required to break the fibers ("breaking force") is determined. Average breaking forces of specific cotton varieties can be found in the Examples.

"Chromosome A05", as used herein, refers to chromosome A05 (numbering according to Wang et al., 2006, Theor Appl Genet. 113(1):73-80) in an A genome diploid *Gossypium* plant, such as *Gossypium herbaceum* or *Gossypium arboreum*, or in an AD allotetraploid *Gossypium* plant, such as *Gossypium hirsutum, Gossypium barbadense* and *Gossypium darwinii*. In one embodiment, the *Gossypium* plant is an A genome diploid *Gossypium* plant comprising 13 A genome chromosome pairs, numbered A01 to A13 according to Wang et al. (2006, Theor Appl Genet. 113(1):73-80), such as *Gossypium herbaceum* or *Gossypium arboreum*. In another embodiment, the *Gossypium* plant is an AD genome allotetraploid *Gossypium* plant comprising 13 A genome and 13 D genome chromosome pairs, numbered A01 to A13 and D01 to D13, respectively, according to Wang et al. (supra), such as *Gossypium hirsutum, Gossypium barbadense* and *Gossypium darwinii.*

In one embodiment, the non-naturally occurring *Gossypium* plant is a *Gossypium hirsutum*, a *Gossypium herbaceum* or a *Gossypium arboreum* plant, preferably a *Gossypium hirsutum* plant, and the superior allele of the fiber strength locus is derived from *Gossypium barbadense.*

*Gossypium barbadense*, in particular *Gossypium barbadense* cv. Pima S7, seeds are publicly available and can be obtained for example from the Cotton Collection (USDA, ARS, Crop Germplasm Research, 2765 F&B Road, College Station, Tex. 77845 on the World Wide Web at ars.grin.gov).

The term "superior allele" of the fiber strength locus refers herein to an allele of the fiber strength locus the presence of which in the genome of a fiber-producing plant results in a higher fiber strength compared to the fiber strength in such fiber-producing plant not comprising the superior allele (i.e., comprising a non-superior allele).

As used herein, the term "allele(s)" means any of one or more alternative forms of a gene or a marker at a particular locus or of a quantitative trait locus (QTL). In a diploid or allotetraploid (amphidiploid) cell of an organism, alleles of a given gene, marker or QTL are located at a specific location or locus (loci plural) on a chromosome. One allele is present on each chromosome of the pair of homologous chromosomes. As used herein, the term "homologous chromosomes" means chromosomes that contain information for the same biological features and contain the same genes or markers at the same loci and the same quantitative trait loci but possibly different alleles of those genes, markers or quantitative trait loci. Homologous chromosomes are chromosomes that pair during meiosis. "Non-homologous chromosomes", representing all the biological features of an organism, form a set, and the number of sets in a cell is called ploidy. Diploid organisms contain two sets of non-homologous chromosomes, wherein each homologous chromosome is inherited from a different parent. In allotetraploid (amphidiploid) species, like cotton, essentially two sets of diploid genomes exist, whereby the chromosomes of the two genomes are referred to as "homeologous chromosomes" (and similarly, the genes, markers and loci of the two genomes are referred to as homeologous genes, markers or loci). A diploid, or allotetraploid (amphidiploid), plant species may comprise a large number of different alleles at a particular locus.

The term "ortholog" of a gene or protein or QTL refers herein to the homologous gene or protein or QTL found in another species, which has the same function as the gene or protein or QTL, but is (usually) diverged in sequence from the time point on when the species harboring the genes or quantitative trait loci diverged (i.e. the genes or quantitative trait loci evolved from a common ancestor by speciation). Orthologs of, e.g., the *Gossypium barbadense* GLUC genes or fiber strength locus may thus be identified in other plant species (e.g. *Gossypium arboreum, Gossypium darwinii*, etc.) based on both sequence comparisons (e.g. based on percentages sequence identity over the entire sequence or over specific domains) and/or functional analysis.

In one embodiment, the superior allele of the fiber strength locus is obtainable from *Gossypium barbadense*, in particular *Gossypium barbadense* cv. PimaS7, i.e. the presence of the *Gossypium barbadense* fiber strength allele in a *Gossypium* plant, such as a *Gossypium hirsutum* plant, results in an increased fiber strength compared to the fiber strength in the *Gossypium* plant, such as the *Gossypium hirsutum* plant, not comprising the *Gossypium barbadense* allele, but, for example, the *Gossypium hirsutum* allele.

In still another embodiment, the *Gossypium barbadense* fiber strength allele is located on chromosome A05 of *Gossypium barbadense* between AFLP marker P5M50-M126.7 and SSR marker CIR280. In another embodiment, the *Gossypium barbadense* fiber strength allele is located on chromosome A05 of *Gossypium barbadense* between AFLP marker P5M50-M126.7 and SSR marker BNL3992. In yet another embodiment, the *Gossypium barbadense* allele is located on chromosome A05 of *Gossypium barbadense* between AFLP marker P5M50-M126.7 and SSR marker CIR401c. In a further embodiment, the LOD peak of the fiber strength QTL allele of *Gossypium barbadense* is located between SSR marker NAU861 or the GLUC1.1 marker and SSR marker CIR401c, in particular at about 0 to 5 cM, more specifically at about 4 cM, especially at about 4.008 cM, from SSR marker NAU861 or the GLUC1.1 marker and at about 0 to 12 cM, more specifically at about 10 cM, especially at about 10.52 cM, from SSR marker CIR401c.

A "(genetic or molecular) marker", as used herein, refers to a polymorphic locus, i.e. a polymorphic nucleotide (a so-called single nucleotide polymorphism or SNP) or a polymorphic DNA sequence at a specific locus. A marker refers to a measurable, genetic characteristic with a fixed position in the genome, which is normally inherited in a Mendelian fashion, and which can be used for mapping of a trait of interest. For example, the fiber strength trait was mapped on chromosome A05 of *Gossypium barbadense* between, amongst others, markers P5M50-M126.7 and CIR280, P5M50-M126.7 and BNL3992, P5M50-M126.7 and CIR401, and linked to markers NAU861, GLUC1.1, and others, as indicated, e.g., in Table 6 in the Examples. Thus, a genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change, i.e. a single nucleotide polymorphism or SNP, or a long DNA sequence, such as microsatellites or Simple Sequence Repeats (SSRs). The nature of the marker is dependent on the molecular analysis used and can be detected at the DNA, RNA or protein level. Genetic mapping can be performed using molecular markers such as, but not limited to, RFLP (restriction fragment length polymorphisms; Botstein et al. (1980), Am J Hum Genet. 32:314-331; Tanksley et al. (1989), Bio/Technology 7:257-263), RAPD [random amplified polymorphic DNA; Williams et al. (1990), NAR 18:6531-6535], AFLP [Amplified Fragment Length Polymorphism; Vos et al. (1995) *NAR* 23:4407-4414], SSRs or microsatellites [Tautz et al. (1989), *NAR* 17:6463-6471]. Appropriate primers or probes are dictated by the mapping method used.

The term "AFLP®" (AFLP® is a registered trademark of KeyGene N.V., Wageningen, The Netherlands), "AFLP analysis" and "AFLP marker" is used according to standard terminology [Vos et al. (1995), *NAR* 23:4407-4414; EP0534858; on the World Wide Web at keygene.com/keygene/techs-apps]. Briefly, AFLP analysis is a DNA fingerprinting technique which detects multiple DNA restriction fragments by means of PCR amplification. The AFLP technology usually comprises the following steps: (i) the restriction of the DNA with two restriction enzymes, preferably a hexa-cutter and a tetra-cutter, such as EcoRI, PstI and MseI; (ii) the ligation of double-stranded adapters to the ends of the restriction fragments, such as EcoRI, PstI and MseI adaptors; (iii) the amplification of a subset of the restriction fragments using two primers complementary to the adapter and restriction site sequences, and extended at their 3' ends by one to three "selective" nucleotides, i.e., the selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. AFLP primers thus have a specific sequence and each AFLP primer has a specific code (the primer codes and their sequences can be found at the Keygene website keygene.com/keygene/pdf/PRIMERCO.pdf; (iv) gel electrophoresis of the amplified restriction fragments on denaturing slab gels or capillaries; (v) the visualization of the DNA fingerprints by means of autoradiography, phospho-imaging, or other methods. Using this method, sets of restriction fragments may be visualized by PCR without knowledge of nucleotide sequence. An AFLP marker, as used herein, is a DNA fragment of a specific size, which is generated and visualized as a band on a gel by carrying out an AFLP analysis. Each AFLP marker is designated by the primer combination used to amplify it, followed by the approximate size (in base pairs) of the amplified DNA fragment, e.g. P5M50-M126.7 refers to AFLP primer combination P05 (or Keygene code P11, which is a PstI primer with additional nucleotides AA; see Table 2) and M50 (which is a MseI primer with additional nucleotides CAT; see Table 2), the use of which in *Gossypium barbadense* results in an amplified DNA fragment of 126.7 bp (see Table 2). It is understood that the size of these fragments may vary slightly depending on laboratory conditions and equipment used. Every time reference is made herein to an AFLP marker by referring to a primer combination and the specific size of a fragment, it is to be understood that such size is approximate, and comprises or is intended to include the slight variations observed in different labs. Each AFLP marker represents a certain locus in the genome.

The term "SSR" refers to Simple Sequence Repeats or microsatellite [Tautz et al. (1989), *NAR* 17:6463-6471]. Short Simple Sequence stretches occur as highly repetitive elements in all eukaryotic genomes. Simple sequence loci usually show extensive length polymorphisms. These simple sequence length polymorphisms (SSLP) can be detected by polymerase chain reaction (PCR) analysis and be used for identity testing, population studies, linkage analysis and genome mapping. "SSR marker", as used herein, refers to markers indicated as CIRx, NAUx and BNLx (wherein x is a number) that are publicly available markers which are used to create genetic maps of different *Gossypium* species (see Cotton Microsatellite Database on the World Wide Web at cottonmarket.org).

A "(genetic or molecular) marker", such as an AFLP or SSR marker, can be dominant (homozygous and heterozygous individuals are not distinguishable) or co-dominant (distinguishing homozygous and heterozygous individuals, e.g., by band intensity), as exemplified in Table 2 below. A "(genetic or molecular) marker", such as an AFLP or SSR marker, can be linked to a gene or locus in "coupling phase" or in "repulsion phase'. For example, a dominant marker linked in coupling to a gene or locus is present in individuals with the gene or locus and absent in individuals without the gene or locus, while a dominant marker linked in repulsion phase to a gene or locus is absent in individuals with the gene or locus and present in individuals without the gene or locus.

Different alleles of markers can exist in different plant species. "*Gossypium barbadense* or *Gossypium hirsutum* alleles of markers linked to the fiber strength locus", as used herein, refers to a form of a marker that is derived from and specific for *Gossypium barbadense* or *Gossypium hirsutum*, respectively. Table 2 examplifies how different alleles of different markers can be identified or distinghuished: column 1 indicates different marker loci on chromosome A05 of *Gossypium barbadense* and/or *Gossypium hirsutum*, column 2 indicates for each marker locus a specific primer pair that can be used to identify the presence or absence of the specific marker locus, column 3 indicates whether a specific marker allele of *Gossypium barbadense* (in particular cv. Pima S7; indicated as 'Pima') and *Gossypium hirsutum* (in particular cv. FM966; indicated as 'FM') generates an amplified DNA fragment and, if so, the size of the amplified DNA fragment, column 4 indicates whether the marker indicated in column 1 is a dominant or a codominant marker as defined above.

TABLE 2

Detection of specific *Gossypium barbadense* or *Gossypium hirsutum* alleles of markers on chromosome A05

| Marker locus on chromosome A05 | Primer pair: | | Amplified fragment (in bp) from FM | Amplified fragment (in bp) From Pima | Codominant/dominant marker |
|---|---|---|---|---|---|
| P5M50-M126.7 | P5 | 5' GACTGCGTACATGCAGAA 3' (SEQ ID NO: 43) | — | 126.7 | dominant |
| | M50 | 5' GATGAGTCCTGAGTAACAT 3' (SEQ ID NO: 44) | | | |
| GLUC1.1A-SNP2 | forward | 5' TAT CCC TCT CGA TGA GTA CGA C 3' (SEQ ID NO: 37) | 134 | 143 | codominant |
| | reverse | 5'CCC AAT GAT GAT GAA CCT GAA TTG3' (SEQ ID NO: 38) | | | |
| NAU861 | forward | 5' CCAAAACTTGTCCCATTAGC 3' (SEQ ID NO: 45) | 205-210 | 215-220 | codominant |
| | reverse | 5' TTCATCTGTTGCCAGATCC 3' (SEQ ID NO: 46) | | | |
| CIR401c | forward | 5' TGGCGACTCCCTTTT 3' (SEQ ID NO: 47) | — | 245-250 | dominant |
| | reverse | 5' AAAAGATGTTACACACACACAC 3' (SEQ ID NO: 48) | | | |
| CIR401b | forward | 5' TGGCGACTCCCTTTT 3' (SEQ ID NO: 47) | 255 | — | dominant |
| | reverse | 5' AAAAGATGTTACACACACACAC 3' (SEQ ID NO: 48) | | | |
| BNL3992 | forward | 5' CAGAAGAGGAGGAGGTGGAG 3' (SEQ ID NO: 49) | 160-165/ 85-90 | 140-145 | codominant |
| | reverse | 5' TGCCAATGATGGAAAACTCA 3' (SEQ ID NO: 50) | | | |
| CIR280 | forward | 5' ACTGCGTTCATTACACC 3' (SEQ ID NO: 51) | — | 205 | dominant |
| | reverse | 5' GCTTCACCCATTCATC 3' (SEQ ID NO: 52) | | | |

As indicated above, the location of the *Gossypium barbadense* fiber strength allele on chromosome A05 can be determined by linked AFLP and/or SSR markers, such as AFLP marker P5M50-M126.7, and SSR markers BNL3992, CIR401b and NAU861. However, it is understood that these AFLP and SSR markers can be converted into other types of molecular markers. When referring to a specific (molecular or genetic) marker in the present invention, it is understood that the definition encompasses other types of molecular markers used to detect the genetic variation originally identified by the AFLP and SSR markers. For example, if an AFLP marker is converted into another molecular marker using known methods, this other marker is included in the definition. For example, AFLP markers can be converted into sequence-specific markers such as, but not limited to STS (sequenced-tagged-site) or SCAR (sequence-characterized-amplified-region) markers using standard technology as described in Meksem et al. [(2001), *Mol Gen Genomics* 265(2):207-214], Negi et al. [(2000), *TAG* 101:146-152], Barret et al. (1989), *TAG* 97:828-833], Xu et al. [(2001), *Genome* 44(1):63-70], Dussel et al. [(2002), *TAG* 105:1190-1195] or Guo et al. [(2003), *TAG* 103:1011-1017]. For example, Dussel et al. [(2002), *TAG* 105:1190-1195] converted AFLP markers linked to resistance into PCR-based sequence tagged site markers such as indel (insertion/deletion) markers and CAPS (cleaved amplified polymorphic sequence) markers.

The conversion of an AFLP marker into an STS marker, for example, generally involves the purification of the DNA fragment from the AFLP gel and the cloning and sequencing of the DNA fragment. Cloning and sequencing of AFLP fragments (bands) can be carried out using known methods [Guo et al. *TAG* 103:1011-1017]. Based on the marker sequence (internal) locus specific PCR primers can be developed [Paran and Michelmore (1993), *TAG* 85:985-993], which amplify fragments of different sizes or wherein the PCR product is cleaved with a restriction enzyme after amplification to reveal a polymorphism. As internal PCR primers often do not reveal polymorphisms related to the EcoRI, MseI or PstI (or other enzymes) restriction site differences, inverse PCR [Hartl and Ochmann (1996), In: Harwood A, editor, *Methods in molecular biology vol 58: basic DNA and RNA protocols*, Humana Press, Totowa N.J. pp 293-301] or PCR-walking [Negi et al. (2000), *TAG* 101:146-152; Siebert et al, (1995), *NAR* 23:1087-1088] may be used to identify flanking sequences, which can then be used to generate simple, locus specific, PCR based markers. Primers can easily be designed using computer software programs such as provided by Sci-Ed (Scientific & Educational Software PO Box 72045, Durham, N.C. 27722-2045 USA). The polymorphism of the STS marker can be detected by gel electrophoresis, or can be detected using fluorometric assays, such as TaqMan® technology (Roche Diagnostics).

In another embodiment, the fiber strength QTL allele of *Gossypium barbadense* comprises at least one *Gossypium barbadense* ortholog of a nucleotide sequence comprised in the genomic DNA sequence spanning the *Gossypium hirsutum* GLUC1.1A gene represented in SEQ ID NO: 53 (see FIG. **9** and the sequence listing).

In another embodiment, the fiber strength QTL allele of *Gossypium barbadense* comprises at least a GLUC1.1 gene encoding a non-functional GLUC1.1 protein as further described below. In one aspect the *Gossypium barbadense* GLUC1.1 gene is located at about 0 to 5 cM, more specifically at about 4 cM, from the LOD peak of the fiber strength QTL allele of *Gossypium barbadense*. In another aspect the *Gossypium barbadense* GLUC1.1 gene is located at about 0 to 2 cM, at about 0 to 1 cM, more specifically at about 0.008 cM of the NAU861 marker located in the fiber strength QTL allele of *Gossypium barbadense*.

In another embodiment, the non-naturally occurring *Gossypium* plant is a *Gossypium hirsutum, Gossypium barbadense*, a *Gossypium herbaceum* or a *Gossypium arboreum* plant, preferably a *Gossypium hirsutum* plant, and wherein the superior fiber strength allele is derived from *Gossypium darwinii*. In one aspect, the fiber strength QTL allele of *Gossypium darwinii* comprises at least a GLUC1.1 gene as further described below.

In still another embodiment, the non-naturally occurring *Gossypium* plant is a *Gossypium hirsutum, Gossypium barbadense* or a *Gossypium herbaceum* plant, preferably a *Gossypium hirsutum* plant, and wherein the superior fiber strength allele is derived from *Gossypium arboreum*. In one aspect, the fiber strength QTL allele of *Gossypium arboreum* comprises at least a GLUC1.1 gene as further described below.

In a particular embodiment, the callose content of the fibers of the non-naturally occurring *Gossypium* plant is increased compared to the callose content of the fibers of an equivalent *Gossypium* plant that does not comprise the at least one superior allele of the fiber strength locus.

"Callose" refers to a plant polysaccharide that comprises glucose residues linked together through beta-1,3-linkages, and is termed a beta-glucan. It is thought to be manufactured at the cell wall by callose synthases and is degraded by beta-1,3-glucanases. The callose content of fibers can be measured by staining the fibers with aniline blue, a dye specific for 1,3-beta-glucans. Under UV, callose deposits present an intense yellow-green fluorescence. Images are analyzed and the ratio Green/Blue is used as a measure for callose. "Cellulose" is the major structural polysaccharide of higher plant cell walls. Chains of beta-1,4-linked glucosyl residues assemble soon after synthesis to form rigid, chemically resistant microfibrils. Their mechanical properties together with their orientation in the wall influence the relative expansion of cells in different directions and determine many of the final mechanical properties of mature cells and organs.

In a particular embodiment, the strength of the fibers of the non-naturally occurring *Gossypium* plant is increased compared to the strength of the fibers of an equivalent *Gossypium* plant that does not comprise the at least one superior allele of the fiber strength locus.

"Increase in fiber strength", as used herein, refers to an average strength of fibers of a specific fiber-producing plant species, such as cotton, which is significantly higher than the average strength of fibers of that specific plant species normally observed. Fiber strength is largely determined by variety. However, it may be affected by plant nutrient deficiencies and weather.

In one aspect of this embodiment, the non-naturally occurring *Gossypium* plant is a *Gossypium hirsutum* plant which is homozygous for the *Gossypium barbadense* fiber strength allele. In a further aspect of this embodiment, the strength of the fibers of the *Gossypium* plant is on average between about 5% and about 10%, more specifically about 7.5%, higher than the fiber strength of a *Gossypium hirsutum* plant which is homozygous for the *Gossypium hirsutum* fiber strength allele. In still a further aspect of this embodiment, the strength of the fibers of the *Gossypium* plant is on average between about 1.6 g/tex and about 3.3 g/tex, more specifically about 2.5 g/tex higher than the fiber strength of a *Gossypium hirsutum* plant which is homozygous for the *Gossypium hirsutum* fiber strength allele. In yet a further aspect of this embodiment, the strength of the fibers of the *Gossypium* plant is on average between about 34.6 g/tex and about 36.3 g/tex, more specifically about 35.5 g/tex, as compared to a fiber strength of on average between about 32.2 g/tex and about 33.8 g/tex, more specifically about 33.0 g/tex of a *Gossypium hirsutum* plant which is homozygous for the *Gossypium hirsutum* fiber strength allele.

A "variety" (abbreviated as var.) or "cultivar" (abbreviated as cv.) is used herein in conformity with the UPOV convention and refers to a plant grouping within a single botanical taxon of the lowest known rank, which grouping can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, can be distinguished from any other plant grouping by the expression of at least one of the said characteristics and is considered as a unit with regard to its suitability for being propagated unchanged (stable).

As used herein, the term "heterozygous" means a genetic condition existing when two different alleles reside at a specific locus, but are positioned individually on corresponding pairs of homologous chromosomes in the cell. Conversely, as used herein, the term "homozygous" means a genetic condition existing when two identical alleles reside at a specific locus, but are positioned individually on corresponding pairs of homologous chromosomes in the cell.

A "fiber-producing plant" refers to a plant species that produces fibers as defined above, such as a cotton plant. Of the *Gossypium* species, the A genome diploid *Gossypium* species and AD genome allotetraploid *Gossypium* species are known to produce spinnable fiber. Botanically, there are three principal groups of cotton that are of commercial importance. The first, *Gossypium hirsutum* (AADD), is native to Mexico and Central America and has been developed for extensive use in the United States, accounting for more than 95% of U.S. production. This group is known in the United States as American Upland cotton, and their fibers vary in length from about ⅞ to about 1 5/16 inches (about 22-about 33 mm). Worldwide it accounts for about 90% of the cotton production. A second botanical group, *G. barbadense* (AADD), which accounts for about 5% of U.S. production and about 8% of the worldwide production, is of early South American origin. With fibers varying in length from about 1¼ to about 1 9/16 inches (about 32-about 40 mm), it is known in the United States as American Pima, but is also commonly referred to as Extra Long Staple (ELS) cotton. A third group, *G. herbaceum* (AA) and *G. arboreum* (AA), embraces cotton plants with fibers of shorter length, about ½ to about 1 inch (about 13-about 25 mm), that are native to India and Eastern Asia. None from this group is cultivated in the United States.

"Fiber length", as used herein, refers to the average length of the longer one-half of the fibers (upper half mean length). In the US, it is usually reported in 100 ths or 32 nds of an inch (see Table 3; 1 inch is 25.4 mm). It is measured, for example, according to United States Department of Agriculture (USDA) standards by passing a "beard" of parallel fibers through a sensing point. The beard is formed when fibers from a sample of cotton are grasped by a clamp, then combed and brushed to straighten and parallel the fibers. Fiber length is largely determined by variety, but the cotton plant's exposure to extreme temperatures, water stress, or nutrient deficiencies may shorten the length. Excessive cleaning and/or drying at the gin may also result in shorter fiber length. Fiber length affects yarn strength, yarn evenness, and the efficiency of the spinning process. The fineness of the yarn which can be successfully produced from given fibers is also influenced by the length of the fiber.

TABLE 3

Cotton fiber length conversion chart for American Upland and Pima cotton

| American Upland cotton | | | | American Pima cotton | |
|---|---|---|---|---|---|
| inches | 32nds | inches | 32nds | inches | 32nds |
| At least 0.79 | 24 | 1.11-1.13 | 36 | At least 1.20 | 40 |
| 0.80-0.85 | 26 | 1.14-1.17 | 37 | 1.21-1.25 | 42 |
| 0.86-0.89 | 28 | 1.18-1.20 | 38 | 1.26-1.31 | 44 |
| 0.90-0.92 | 29 | 1.21-1.23 | 39 | 1.32-1.36 | 46 |
| 0.93-0.95 | 30 | 1.24-1.26 | 40 | 1.37-1.42 | 48 |
| 0.96-0.98 | 31 | 1.27-1.29 | 41 | 1.43-1.47 | 50 |
| 0.99-1.01 | 32 | 1.30-1.32 | 42 | At least 1.48 | 52 |
| 1.02-1.04 | 33 | 1.33-1.35 | 43 | | |
| 1.05-1.07 | 34 | At least 1.36 | At least 44 | | |
| 1.08-1.10 | 35 | | | | |

Source: on the World Wide Web at cottoninc.com 1 inch = 2.54 cm

An "industrially relevant fiber length", as used herein, refers to a length of fibers of a specific cotton species which is on average at least equal to or not significantly smaller than the length of fibers of that specific cotton variety normally observed. For *G. hirsutum*, an industrially relevant fiber length is reported to vary from about ⅞ to 1$\frac{5}{16}$ inches (about 22-about 33 mm). For *G. barbadense*, an industrially relevant fiber length is reported to vary from 1¼ to 1$\frac{9}{16}$ inches (about 32-about 40 mm). For *G. herbaceum* (AA) and *G. arboreum* (AA), an industrially relevant fiber length is reported to vary from ½ to 1 inch (about 13-about 25 mm).

Whenever reference to a "plant" or "plants" according to the invention is made, it is understood that also plant parts (cells, tissues or organs, seeds, fibers, severed parts such as roots, leaves, flowers, pollen, etc.), progeny of the plants which retain the distinguishing characteristics of the parents (especially the fiber properties), such as seed obtained by selfing or crossing, e.g. hybrid seed (obtained by crossing two inbred parental lines), hybrid plants and plant parts derived there from are encompassed herein, unless otherwise indicated.

The term "fiber strength allele detection assay" refers herein to an assay that indicates (directly or indirectly) the presence or absence of specific alleles of the fiber strength locus of the present invention. In one embodiment it allows one to determine whether a particular fiber strength allele is homozygous or heterozygous at the locus in any individual plant.

In another aspect of the invention, methods are provided for generating and/or selecting *Gossypium* plants, and parts and progeny thereof, comprising at least one superior allele of the fiber strength locus.

In one embodiment, the superior allele of the fiber strength locus is the *Gossypium barbadense* allele and the method comprises the step of identifying a *Gossypium* plant that comprises the *Gossypium barbadense* fiber strength allele based on the presence of *Gossypium barbadense* alleles of markers linked to the fiber strength locus, such as the markers linked to the *Gossypium barbadense* fiber strength allele indicated above and in Table 6 and 13.

In a particular aspect, the method comprises the step of determining the presence of *Gossypium barbadense* alleles of markers linked to the fiber strength locus in the genomic DNA of a plant selected from the group consisting of: AFLP marker P5M50-M126.7, SSR marker CIR280, SSR marker BNL3992, SSR marker CIR401c, SSR marker NAU861, a polymorphic site in a genomic DNA sequence of the plant corresponding to a genomic DNA sequence comprised in SEQ ID NO: 53, and a polymorphic site in a nucleotide sequence of a GLUC1.1A gene in the genomic DNA of the plant corresponding to the nucleotide sequence of a GLUC1.1A gene of SEQ ID NO: 5, such as the SNP markers indicated as GLUC1.1A-SNP2, 3, 5, 6 and 8 below and in Table 13.

In a further embodiment, the superior allele of the fiber strength locus is the *Gossypium darwinii* allele and the method comprises the step of identifying a *Gossypium* plant that comprises the *Gossypium darwinii* fiber strength allele based on the presence of *Gossypium darwinii* alleles of markers linked to the fiber strength locus, such as the markers linked to the *Gossypium darwinii* fiber strength allele indicated above and in Table 13.

In a particular aspect, the method comprises the step of determining the presence of a *Gossypium darwinii* allele of a polymorphic site in a nucleotide sequence of a GLUC1.1A gene in the genomic DNA of the plant corresponding to the nucleotide sequence of a GLUC1.1A gene of SEQ ID NO: 56, such as the SNP markers indicated as GLUC1.1A-SNP2, 3, 5, 6 and 8 below and in Table 13.

In a further embodiment, the superior allele of the fiber strength locus is the *Gossypium arboreum* allele and the method comprises the step of identifying a *Gossypium* plant that comprises the *Gossypium arboreum* fiber strength allele based on the presence of *Gossypium arboreum* alleles of markers linked to the fiber strength locus, such as the markers linked to the *Gossypium arboreum* fiber strength allele indicated above and in Table 13.

In a particular aspect, the method comprises the step of determining the presence of a *Gossypium arboreum* allele of a polymorphic site in a nucleotide sequence of a GLUC1.1A gene in the genomic DNA of the plant corresponding to the nucleotide sequence of a GLUC1.1A gene of SEQ ID NO: 21, such as the SNP marker indicated as GLUC1.1A-SNP7 below and in Table 13.

Markers linked to the fiber strength locus can be used for marker assisted selection (MAS) or map based cloning of the fiber strength locus. MAS involves screening plants for the presence or absence of linked markers. In particular plants are screened for the presence of markers flanking the locus or gene or linked to the locus or gene. Based on the presence/absence of the marker(s) plants are selected or discarded during the breeding program. MAS can significantly speed up breeding programs and introgression of a particular locus or gene into another genetic background, and can also reduce problems with genotype x environment interactions. MAS is also useful in combining different fiber strength loci in one plant. The presence or absence of a specific fiber strength allele, such as the *Gossypium barbadense* fiber strength allele, can be inferred from the presence or absence of molecular markers, such as the AFLP and SSR markers indicated above (see for example Table 2) or markers derived from them, linked to the specific allele. For example, *Gossypium barbadense* plants, in particular *Gossypium barbadense* cv. Pima S7 plants, may be crossed to *Gossypium hirsutum* plants and progeny plants from this cross are then screened for the presence of one or more AFLP and/or SSR markers linked to the *Gossypium barbadense* fiber strength allele, for example, by using the *barbadense* allele identification protocol.

Breeding procedures such as crossing, selfing, and backcrossing are well known in the art [see Allard R W (1960) *Principles of Plant Breeding*. John Wiley & Sons, New York, and Fehr W R (1987) *Principles of Cultivar Development*, Volume 1, Theory and Techniques, Collier Macmillan Publishers, London. ISBN 0-02-949920-8]. Superior alleles of the fiber strength locus, such as the *Gossypium barbadense* fiber strength allele, can be transferred into other breeding lines or varieties either by using traditional breeding methods alone or by using additionally MAS. In traditional breeding methods the increased callose content and/or increased fiber strength phenotype is assessed in the field or in controlled environment tests in order to select or discard plants comprising or lacking the superior fiber strength allele. Different crosses can be made to transfer the superior fiber strength allele, such as the *Gossypium barbadense* fiber strength allele, into lines of other *Gossypium* species or varieties, such as A genome diploid *Gossypium* plant lines, such as *Gossypium herbaceum* or *Gossypium arboreum* plant lines, or in AD allotetraploid *Gossypium* plant lines, such as *Gossypium hirsutum* and *Gossypium barbadense* plant lines, in particularly in *Gossypium barbadense* plant lines different from the Pima S7 variety. The breeding program may involve crossing to generate an F1 (first filial generation), followed by several generations of selfing (generating F2, F3, etc.). The breeding program may also involve backcrossing (BC) steps, whereby the offspring are backcrossed to one of the parental lines (termed the recurrent parent). Breeders select for agronomically important traits, such as high yield, high fiber quality, disease resistance, etc., and develop thereby elite breeding lines (lines with good agronomic characteristics). In addition, plants are bred to comply with fiber quality standards, such as American Pima or American Upland fiber quality.

The "*barbadense* or *hirsutum* allele identification protocol", as used herein, refers to the identification of the *Gossypium barbadense* and/or *Gossypium hirsutum* allele of the fiber strength locus comprising the steps of: extracting DNA from plant tissue such as leaf tissue or seeds and carrying out an analysis of linked markers, such as an AFLP and/or SSR analysis for one or more of the linked AFLP and/or SSR markers, using, for example, specific primer pairs to identify the *barbadense* or *hirsutum* allele, such as those indicated in Table 2. The *barbadense* or *hirsutum* allele identification protocol may be carried out on DNA obtained from individual plants or on DNA obtained from bulks (or pools). In one embodiment kits for detecting the presence of the *Gossypium barbadense* and/or *Gossypium hirsutum* fiber strength allele in *Gossypium* DNA are provided. Such a kit comprises, for example, primers or probes able to detect a DNA marker, such as an AFLP and/or an SSR marker, linked to the *Gossypium barbadense* and/or *Gossypium hirsutum* fiber strength allele. The kit may further comprise samples, which can be used as positive or negative controls and additional reagents for AFLP and/or SSR analysis. The samples may be tissue samples or DNA samples. As positive control may, for example, *Gossypium barbadense* seeds, in particular from cv. Pima S7, be included. As negative controls may, for example, *Gossypium hirsutum* seeds, in particular from cv. FM966, be included.

In a further aspect, methods are provided to distinguish between the presence of superior and non-superior alleles of the fiber strength locus. In one embodiment, methods are provided to distinguish between the presence of the *Gossypium barbadense* allele and the *Gossypium hirsutum* allele comprising the step of determining the presence of *Gossypium barbadense* and/or *Gossypium hirsutum* alleles of markers linked to the fiber strength locus, such as the markers linked to the fiber strength locus indicated above, for example, those indicated in Table 2 and Table 13.

Thus, in one embodiment, a method is provided for distinguishing between the presence of the *Gossypium barbadense* and *Gossypium hirsutum* fiber strength alleles by determining the presence of *Gossypium barbadense* and *Gossypium hirsutum* alleles of markers linked to the fiber strength locus in the genomic DNA of a plant selected from the group consisting of: AFLP marker P5M50-M126.7, SSR marker CIR280, SSR marker BNL3992, SSR marker CIR401, SSR marker NAU861, a polymorphic site in a genomic DNA sequence of the plant corresponding to a genomic DNA sequence comprised in SEQ ID NO: 53, and a polymorphic site in a nucleotide sequence of a GLUC1.1A gene in the genomic DNA of the plant corresponding to the nucleotide sequence of a GLUC1.1A gene of SEQ ID NO: 5, such as the SNP markers indicated as GLUC1.1A-SNP2, 3, 5, 6 and 8 below and in Table 13.

According to another aspect of the invention, methods are provided for altering the callose content of a fiber in a *Gossypium* plant, particularly increasing the callose content of a fiber, comprising the step of introgressing a superior allele of the cotton fiber strength locus on chromosome A05, such as the *Gossypium barbadense* allele, in the *Gossypium* plant.

According to yet another aspect of the invention, methods are provided for altering the properties of a fiber in a *Gossypium* plant, particularly increasing the strength of a fiber, comprising the step of introgressing a superior allele of the cotton fiber strength locus on chromosome A05, such as the *Gossypium barbadense* allele, in the *Gossypium* plant.

The current invention is further based on the unexpected finding that the functionality and the timing of expression of the GLUC1.1A gene, which was located in the support interval of the strength locus, differ between *G. hirsutum* and *G. barbadense*. It was found that, while *G. hirsutum* plants comprise a GLUC1.1A gene which is functionally expressed during the fiber strength building stage of fiber development, more particularly during the fiber maturation phase, *G. barbadense* plants comprise a GLUC1.1A gene which is non-functionally expressed during the fiber strength building phase. The GLUC1.1D gene on the other hand is functionally expressed during the entire fiber strength building stage in both *Gossypium* species. It was further found that addition of exogenous endo-1,3-beta-glucanase to fibers of *Gossypium barbadense* reduces the callose content and the strength of the fibers. Based on these findings, it is believed that the renown strength of the fibers of *G. barbadense* might be, at least in part, caused by a higher callose content in the fibers and that this higher callose content might be caused by the absence of a functionally expressed A subgenome-specific fiber-specific endo-1,3-beta-glucanase gene. It is further believed that by abolishing the functional expression of specific alleles of GLUC genes during the fiber strength building stage in fiber-producing plants while maintaining the functional expression of specific other GLUC genes during the fiiber strength building stage, it is possible to fine tune the amount and/or type of functional GLUC proteins produced during the fiber strength building stage, thus influencing the degradation of callose in the fiber which in turn influences the strength and length of the fiber produced. It is believed that the absolute and relative amount of different GLUC proteins in fibers can thus be tuned in such a way so as to attain a proper balance between fiber length and strength.

Thus, in a further aspect, the present invention provides a non-naturally occurring fiber-producing plant, and parts and progeny thereof, characterized in that the functional expression of at least one allele of at least one fiber-specific GLUC gene that is functionally expressed during the fiber strength building phase, in particular during the maturation phase of fiber development, is abolished.

The term "gene" means a DNA sequence comprising a region (transcribed region), which is transcribed into an RNA molecule (e.g. into a pre-mRNA, comprising intron sequences, which is then spliced into a mature mRNA, or directly into a mRNA without intron sequences) in a cell, operable linked to regulatory regions (e.g. a promoter). A gene (genomic DNA) may thus comprise several operably linked sequences, such as a promoter, a 5' leader sequence comprising e.g. sequences involved in translation initiation, a (protein) coding region (with introns) and a 3' non-translated sequence comprising e.g. transcription termination sites. "cDNA sequence" refers to a nucleic acid sequence comprising the 5' untranslated region, the coding region without introns and the 3' untranslated region and a polyA tail. "Endogenous gene" is used to differentiate from a "foreign gene", "transgene" or "chimeric gene", and refers to a gene from a plant of a certain plant genus, species or variety, which has not been introduced into that plant by transformation (i.e. it is not a "transgene"), but which is normally present in plants of that genus, species or variety, or which is introduced in that plant from plants of another plant genus, species or variety, in which it is normally present, by normal breeding techniques or by somatic hybridization, e.g., by protoplast fusion. Similarly, an "endogenous allele" of a gene is not introduced into a plant or plant tissue by plant transformation, but is, for example, generated by plant mutagenesis and/or selection, introgressed from another plant species by, e.g., marker-assisted selection, or obtained by screening natural populations of plants.

"Expression of a gene" or "gene expression" refers to the process wherein a DNA region, which is operably linked to appropriate regulatory regions, particularly a promoter, is transcribed into an RNA molecule. The RNA molecule is then processed further (by post-transcriptional processes) within the cell, e.g. by RNA splicing and translation initiation and translation into an amino acid chain (polypeptide), and translation termination by translation stop codons. The term "functionally expressed" is used herein to indicate that a functional, i.e. biologically active, protein is produced; the term "not functionally expressed" to indicate that a protein with significantly reduced or no functionality (biological activity) is produced or that no or a significantly reduced amount of protein is produced.

The term "fiber specific" or "fiber cell specific", with respect to the expression of a gene, refers to, for practical purposes, the highly specific, expression of a gene in fiber cells of plants, such as cotton plants. In other words, transcript levels of a DNA in tissues different of fiber cells is either below the detection limit or very low (less than about 0.2 picogram per microgram total RNA).

The term "fiber strength building phase" commonly refers herein to the secondary cell wall synthesis and maturation phase of fiber development as defined above.

The term "GLUC gene" refers herein to a nucleic acid sequence encoding an endo-1,3-beta-glucanase (GLUC) protein.

The term "nucleic acid sequence" (or nucleic acid molecule) refers to a DNA or RNA molecule in single or double stranded form, particularly a DNA encoding a protein or protein fragment according to the invention. An "endogenous nucleic acid sequence" refers to a nucleic acid sequence within a plant cell, e.g. an endogenous (allele of a) GLUC gene present within the nuclear genome of a plant cell. An "isolated nucleic acid sequence" is used to refer to a nucleic acid sequence that is no longer in its natural environment, for example in vitro or in a recombinant bacterial or plant host cell.

The terms "protein" and "polypeptide" are used interchangeably and refer to molecules consisting of a chain of amino acids, without reference to a specific mode of action, size, 3-dimensional structure or origin. A "fragment" or "portion" of a protein may thus still be referred to as a "protein". An "isolated protein" is used to refer to a protein that is no longer in its natural environment, for example in vitro or in a recombinant bacterial or plant host cell. "Amino acids" are the principal building blocks of proteins and enzymes. They are incorporated into proteins by transfer RNA according to the genetic code while messenger RNA is being decoded by ribosomes. During and after the final assembly of a protein, the amino acid content dictates the spatial and biochemical properties of the protein or enzyme. The amino acid backbone determines the primary sequence of a protein, but the nature of the side chains determines the protein's properties. "Similar amino acids", as used herein, refers to amino acids that have similar amino acid side chains, i.e. amino acids that have polar, non-polar or practically neutral side chains. "Non-similar amino acids", as used herein, refers to amino acids that have different amino acid side chains, for example an amino acid with a polar side chain is non-similar to an amino acid with a non-polar side chain. Polar side chains usually tend to be present on the surface of a protein where they can interact with the aqueous environment found in cells ("hydrophilic" amino acids). On the other hand, "non-polar" amino acids tend to reside within the center of the protein where they can interact with similar non-polar neighbors ("hydrophobic" amino acids"). Examples of amino acids that have polar side chains are arginine, asparagine, aspartate, cysteine, glutamine, glutamate, histidine, lysine, serine, and threonine (all hydrophilic, except for cysteine which is hydrophobic). Examples of amino acids that have non-polar side chains are alanine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, and tryptophan (all hydrophobic, except for glycine which is neutral).

An "enzyme" is a protein comprising enzymatic activity, such as functional, i.e. biologically active, endo-1,3-beta-glucanase or glucan endo-1,3-beta-D-glucosidase (GLUC) proteins (EC 3.2.1.39). GLUC proteins belong to the glycosyl hydrolase family 17 (GH17) enzyme grouping and are capable of hydrolyzing 1,3-beta-D-glucosidic linkages in 1,3-beta-D-glucans, including long chain 1,3-beta-D-glucans called callose (see also on the World Wide Web at cazy.org/fam/GH17. The GH17 group is identified by the following amino acid recognition signature: [LIVMKS]-X-[LIVMFYWA](3)-[STAG]-E-[STACVI]-G-[WY]*-P-[STN]-X-[SAGQ], where E, such as Glu249 in GhGLUC1.1A (SEQ ID NO: 2 and 4) and similar or identical amino acids in other GLUC1.1 proteins (for example as indicated in FIG. **7**), is an active site residue. The GH17 recognition signal of GLUC1.1 enzymes, as described herein, further contains a conserved tryptophan (W) residue at the position indicated with *, such as Trp252 in GhGLUC1.1A (SEQ ID NO: 2 and 4) and similar or identical amino acids in other GLUC1.1 proteins (for example as indicated in FIG. **7**), which is predicted to be involved in the interaction with the glucan substrate.

In one embodiment, the fiber-specific GLUC gene that is functionally expressed during the fiber strength building phase, is a GLUC1.1 gene.

The term "GLUC1.1 gene" refers herein to a nucleic acid sequence encoding a GLUC1.1 protein. In particular, a "GLUC1.1 gene", as used herein, refers to a GLUC gene encoding a cDNA sequence with at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, 100% sequence identity to SEQ ID NO: 3 or comprises a coding sequence with at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, 100% sequence identity to the nucleotide at position 2410 to the nucleotide at position 3499 of SEQ ID NO: 1.

A "GLUC1.1 protein", as used herein, refers to a GLUC protein that has at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, 1000% sequence identity to SEQ ID NO: 4.

A functional "GLUC1.1 protein", as used herein, refers to a GLUC1.1 protein that is capable of hydrolyzing 1,3-beta-D-glucosidic linkages in 1,3-beta-D-glucans, that has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 4 and that comprises amino acid residues similar to the active site residues of the GLUC1.1 protein of SEQ ID NO:4. A non-functional "GLUC1.1 protein", as used herein, refers to a GLUC1.1 protein that is not capable of hydrolyzing 1,3-beta-D-glucosidic linkages in 1,3-beta-D-glucans. In particular, a non-functional GLUC1.1 protein lacks one or more amino acid residues similar to the active site residues of the GLUC1.1 protein of SEQ ID NO:4.

An "active site" or "catalytic site", as used herein, refers to a position on the three-dimensional structure of an enzyme which is involved in substrate binding, such as binding of 1,3-beta-D-glucans to GLUC enzymes, and in the biological activity of the enzyme, such as the hydrolyzation of 1,3-beta-D-glucosidic linkages in 1,3-beta-D-glucans of GLUC enzymes. "Active site (amino acid) residues", as used herein, refer to amino acid residues that are located within the active site of an enzyme and play a crucial role in substrate binding or in enzyme activity. A "glycosylation site", as used herein, refers to a position on the three-dimensional structure of an enzyme which is glycosylated, i.e. a site to which (branched) oligosaccharides bind which may function in increasing stability, such as thermostability, of the protein. "Glycosylation site (amino acid) residues", as used herein, refer to amino acid residues within the glycosylation site of an enzyme to which (branched) oligosaccharides bind. Predictions of the three-dimensional structure of the endo-1,3-beta-glucanase enzymes as described herein indicate that the active site and the glycosylation site of the barley 1,3-1,4-beta-glucanase (as described by Müller et al., 1998, J of Biol Chem 273 (6): 3438-3446; called "laq0" in the Protein Data Bank, which is freely available on the World Wide Web at rcsb.org/pdb) are conserved, for example, in the *Gossypium hirsutum* GLUC1.1A and D, the *Gossypium barbadense* GLUC1.1D and the *Gossypium herbaceum* GLUC1.1A proteins as described herein, while the *Gossypium barbadense* GLUC1.1A protein, the *Gossypium darwinii* GLUC1.1A protein, and the *Gossypium arboreum* GLUC1.1A protein as described herein lack most conserved amino acids located within these sites these sites (see, e.g., Table 4, FIG. **3** and Examples). Active site and glycosylation residues in other GLUC1.1 proteins can be determined by aligning the amino acid sequences of the different GLUC1.1 proteins with the GLUC1.1 proteins of the present invention, such as the amino acid sequence of GhGLUC1.1A in SEQ ID NO:4, and identifying identical or similar residues in the other GLUC1.1 proteins.

TABLE 4

Amino acid regions and positions of active site residues and glycosylation site residues in GLUC1.1A and D proteins of the three principal groups of cotton of commercial interest

| GLUC protein: barley 1,3-1,4- | GhGLUC1.1 | | GbGLUC1.1 | | GheGLUC1.1 | GaGLUC1.1 |
|---|---|---|---|---|---|---|
| beta-glucanase | A | D | A | D | A | A |
| SEQ ID NO: | 2/4 | 8/10 | 6/55 | 12/14 | 24 | 22 |
| Protein size (aa) | 325 | 337 | 179 | 337 | 337 | 78 |
| Mature protein | 311 | 311 | 165 | 311 | 311 | 52 |
| aa encoded by exon 1 | 11 | 23 | 11 | 23 | 23 | 23 |
| aa encoded by exon 2 | 314 | 314 | 168 | 314 | 314 | 55 |
| Active site residue | | | | | | |
| Tyr33 | Tyr48 | Tyr60 | Tyr48 | Tyr60 | Tyr60 | Tyr60 |
| Glu232 | Glu249 | Glu261 | — | Glu261 | Glu261 | — |
| | Trp252 | Trp264 | — | Trp264 | Trp264 | — |
| Glu288 | Glu308 | Glu320 | — | Glu320 | Glu320 | — |
| Glycosylation site residue: | | | | | | |
| Asn190 | Asn202 | ND | — | ND | Asn214 | — |

—: not present; ND: not determined

The terms "target peptide", "transit peptide" or "signal peptide" refer to amino acid sequences which target a protein to intracellular organelles. The GLUC1.1 proteins as described herein comprise a signal peptide at their N-terminal end, such as the amino acid sequence indicated before the putative post-translational splicing site in FIGS. **2** and **7**. "Mature protein" refers to a protein without the signal peptide, such as the GLUC1.1 proteins as described herein without the amino acid sequence indicated before the putative post-translational splicing site in FIGS. **2** and **7**. "Precursor protein" or "preproenzyme" refers to the mature protein with its signal peptide.

In another embodiment, the fiber-producing plant is a *Gossypium* plant. In a particular aspect, the *Gossypium* GLUC1.1 allele is a GLUC1.1A or D allele.

A "GLUC1.1A gene", as used herein, refers to a GLUC1.1 gene located on the A subgenome of a *Gossypium* diploid or allotetraploid species ("GLUC1.1A locus") and encoding a GLUC1.1A protein. In particular, a GLUC1.1A gene encodes a cDNA sequence with at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 3 or comprises a coding sequence with at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the nucleotide at position 2410 to the nucleotide at position 3499 of SEQ ID NO: 1. Similarly, a "GLUC1.1D gene", as used herein, refers to a GLUC1.1 gene located on the D subgenome of a *Gossypium* diploid or allotetraploid species ("GLUCHD locus") and encoding a GLUC1.1D protein. In particular, a GLUC1.1D gene encodes a cDNA sequence with at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 9 or comprises a coding sequence with at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the nucleotide at position 3337 to the nucleotide at position 4444 of SEQ ID NO: 7.

A "GLUC1.1A protein", as used herein, refers to a GLUC1.1 protein encoded by a GLUC1.1 gene located on the A subgenome of a *Gossypium* diploid or allotetraploid species and having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 4. Similarly, a "GLUC1.1D protein", as used herein, refers to a GLUC protein encoded by a GLUC1.1 gene located on the D subgenome of a *Gossypium* diploid or allotetraploid species and having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 10.

In another embodiment the fiber-producing plant is a *Gossypium hirsutum* plant. In a particular aspect, the *Gossypium hirsutum* GLUC1.1 allele is a GhGLUC1.1A or a GhGLUC1.1D allele, preferably a GhGLUC1.1A allele.

As described in WO2008/083969, the GLUC1.1A and GLUC1.1D genes of *Gossypium hirsutum* can be distinguished by the presence of a cleaved amplified polymorphic sequence (CAPS) marker using an AlwI restriction enzyme recognition site present in the nucleotide sequence of GhGLUC1.1A that is absent in the nucleotide sequence of GhGLUC1.1D and by their timing of expression: whereas the GhGLUC1.1D is expressed during the entire fiber strength building phase (from about 14 to 17 DPA on depending on growth conditions), onset of GhGLUC1.1A is delayed until the beginning of the late fiber maturation phase (about 30-40 DPA depending on growth conditions). The GLUC1.1A and GLUC1.1D genes of *Gossypium barbadense* can also be distinguished by the presence of the CAPS marker using the AlwI restriction enzyme recognition site present in the nucleotide sequence of GbGLUC1.1A that is absent in the nucleotide sequence of GbGLUC1.1D. Both genes are however expressed during the entire fiber strength building phase (from about 14 to 17 DPA on depending on growth conditions). The level of expression of GbGLUC1.1A is however much lower than the level of expression of GbGLUC1.1D.

In one embodiment, the functional expression of the at least one GLUC allele is abolished by mutagenesis.

"Mutagenesis", as used herein, refers to the process in which plant cells (e.g., *Gossypium* seeds or other parts, such as pollen, etc.) are subjected to a technique which induces mutations in the DNA of the cells, such as contact with a mutagenic agent, such as a chemical substance (such as ethylmethylsulfonate (EMS), ethylnitrosourea (ENU), etc.) or ionizing radiation (neutrons (such as in fast neutron mutagenesis, etc.), alpha rays, gamma rays (such as that supplied by a Cobalt 60 source), X-rays, UV-radiation, etc.), or a combination of two or more of these. Thus, the desired mutagenesis of one or more GLUC alleles may be accomplished by use of chemical means such as by contact of one or more plant tissues with ethylmethylsulfonate (EMS), ethylnitrosourea, etc., by the use of physical means such as x-ray, etc, or by gamma radiation, such as that supplied by a Cobalt 60 source. While mutations created by irradiation are often large deletions or other gross lesions such as translocations or complex rearrangements, mutations created by chemical mutagens are often more discrete lesions such as point mutations. For example, EMS alkylates guanine bases, which results in base mispairing: an alkylated guanine will pair with a thymine base, resulting primarily in G/C to A/T transitions. Following mutagenesis, *Gossypium* plants are regenerated from the treated cells using known techniques. For instance, the resulting *Gossypium* seeds may be planted in accordance with conventional growing procedures and following self-pollination seed is formed on the plants. Additional seed that is formed as a result of such self-pollination in the present or a subsequent generation may be harvested and screened for the presence of mutant GLUC alleles. Several techniques are known to screen for specific mutant alleles, e.g., Deleteagene™ (Delete-a-gene; Li et al., 2001, Plant J 27: 235-242) uses polymerase chain reaction (PCR) assays to screen for deletion mutants generated by fast neutron mutagenesis, TILLING (targeted induced local lesions in genomes; McCallum et al., 2000, Nat Biotechnol 18:455-457) identifies EMS-induced point mutations, etc. Additional techniques to screen for the presence of specific mutant GLUC alleles are described in the Examples below.

"Wild type" (also written "wildtype" or "wild-type"), as used herein, refers to a typical form of a plant or a gene as it most commonly occurs in nature. A "wild type plant" refers to a plant with the most common phenotype of such plant in the natural population. A "wild type allele" refers to an allele of a gene required to produce the wild-type phenotype. By contrast, a "mutant plant" refers to a plant with a different rare phenotype of such plant in the natural population or produced by human intervention, e.g. by mutagenesis, and a "mutant allele" refers to an allele of a gene required to produce the mutant phenotype.

As used herein, the term "wild type GLUC" (e.g. wild type GLUC1.1A or GLUC1.1D), means a naturally occurring GLUC allele found within plants, in particular *Gossypium* plants, which encodes a functional GLUC protein (e.g. a functional GLUC1.1A or GLUC1.1D, respectively). In contrast, the term "mutant GLUC" (e.g. mutant GLUC1.1A or GLUC1.1D), as used herein, refers to a GLUC allele, which does not encode a functional GLUC protein, i.e. a GLUC allele encoding a non-functional GLUC protein (e.g. a non-functional GLUC1.1A or GLUC1.1D, respectively), which, as used herein, refers to a GLUC protein having no biological activity or a significantly reduced biological activity as compared to the corresponding wild-type functional GLUC protein, or encoding no GLUC protein or a significantly reduced amount of GLUC protein. Such a "mutant GLUC allele" is a GLUC allele, which comprises one or more mutations in its nucleic acid sequence, whereby the mutation(s) preferably result in a significantly reduced (absolute or relative) amount of functional GLUC protein in the cell in vivo. As used herein, a "full knock-out GLUC1.1A allele" is a mutant GLUC1.1A allele the presence of which in homozygous state in the plant (e.g. a *Gossypium hirsutum* plant with two full knock-out GLUC1.1A alleles and two wild-type GLUC1.1D alleles) results in an increase of fiber strength in that plant. Mutant alleles of the GLUC protein-encoding nucleic acid sequences are designated as "gluc" (e.g. gluc1.1a or gluc1.1d, respectively) herein. Mutant alleles can be either "natural mutant" alleles, which are mutant alleles found in nature (e.g. produced spontaneously without human application of mutagens), such as the *Gossypium barbadense* GLUC1.1A allele, the *Gossypium darwinii* GLUC1.1A allele, and the *Gossypium arboreum* GLUC1.1A allele, or "induced mutant" alleles, which are induced by human intervention, e.g. by mutagenesis.

Thus in one aspect of the embodiment, GLUC mutant plants are provided herein, whereby the mutant alleles are selected from the GLUC1.1A and/or GLUC1.1D genes. Thus in a particular aspect, the genotype of these GLUC mutant plants can be described as: GLUC1.1A/gluc1.1a; GLUC1.1D/gluc1.1d; GLUC1.1A/gluc1.a, GLUC1.1D/GLUC1.1D; or GLUC1.1A/GLUC1.1A, GLUC1.1D/gluc1.1d.

In a further aspect of the embodiment, homozygous GLUC mutant plants or plant parts are provided, whereby the mutant alleles are selected from the GLUC1.1A and GLUC1.1D genes. Thus in a particular aspect, homozygous GLUC mutant plants are provided herein, wherein the genotype of the plant can be described as: gluc1.1a/gluc1.1a; gluc1.1d/gluc1.1d; gluc1.1a/gluc1.1a, GLUC1.1D/GLUC1.1D or GLUC1.1A/GLUC1.1A, gluc1.1d/gluc1.1d.

In a further aspect of the invention the homozygous GLUC mutant plants or plant parts comprise a further mutant allele, wherein the mutant plants or plant parts are heterozygous for the additional mutant GLUC allele. Thus in a further particular aspect, homozygous GLUC mutant plants comprising one further mutant GLUC allele are provided herein, wherein the genotype of the plant can be described as: GLUC1.1-A/gluc1.1-a, gluc1.1-d/gluc1.1-d or gluc1.1a/gluc1.1a, GLUC1.1D/gluc1.1d.

In another embodiment, the functional expression of the at least one GLUC allele is abolished by introgression of a non-functionally expressed orthologous GLUC allele or of a mutagenized allele of the GLUC gene.

In one aspect of this embodiment, the non-functionally expressed orthologous GLUC allele can be isolated from specific cotton species, for example from *Gossypium barbadense, darwinii* or *arboreum.*

In yet another embodiment, the functional expression of the at least one allele of the GLUC gene is abolished by introduction of a chimeric gene comprises the following operably linked DNA elements:

(a) a plant expressible promoter,
(b) a transcribed DNA region, which when transcribed yields an inhibitory RNA molecule capable of reducing the expression of the GLUC allele, and
(c) a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of the plant.

Several methods are available in the art to produce an inhibitory or a silencing RNA molecule, i.e. an RNA molecule which when expressed reduces the expression of a particular gene or group of genes, including the so-called "sense" or "antisense" RNA technologies.

Thus in one embodiment, the inhibitory RNA molecule encoding chimeric gene is based on the so-called antisense technology. In other words, the coding region of the chimeric gene comprises a nucleotide sequence of at least 19 or 20 consecutive nucleotides of the complement of the nucleotide sequence of the GLUC allele. Such a chimeric gene may be constructed by operably linking a DNA fragment comprising at least 19 or 20 nucleotides from the GLUC allele, isolated or identified as described elsewhere in this application, in inverse orientation to a plant expressible promoter and 3' end formation region involved in transcription termination and polyadenylation.

In another embodiment, the inhibitory RNA molecule encoding chimeric gene is based on the so-called co-suppression technology. In other words, the coding region of the chimeric gene comprises a nucleotide sequence of at least 19 or 20 consecutive nucleotides of the nucleotide sequence of the GLUC allele. Such a chimeric gene may be constructed by operably linking a DNA fragment comprising at least 19 or 20 nucleotides from the GLUC allele, in direct orientation to a plant expressible promoter and 3' end formation region involved in transcription termination and polyadenylation.

The efficiency of the above mentioned chimeric genes in reducing the expression of the GLUC allele may be further enhanced by the inclusion of a DNA element which results in the expression of aberrant, unpolyadenylated inhibitory RNA molecules or results in the retention of the inhibitory RNA molecules in the nucleus of the cells. One such DNA element suitable for that purpose is a DNA region encoding a self-splicing ribozyme, as described in WO 00/01133 (incorporated by reference). Another such DNA element suitable for that purpose is a DNA region encoding an RNA nuclear localization or retention signal, as described in WO03/076619 (incorporated by reference).

A convenient and very efficient way of downregulating the expression of a gene of interest uses so-called double-stranded RNA (dsRNA) or interfering RNA (RNAi), as described e.g. in WO99/53050 (incorporated by reference). In this technology, an RNA molecule is introduced into a plant cell, whereby the RNA molecule is capable of forming a double stranded RNA region over at least about 19 to about 21 nucleotides, and whereby one of the strands of this double stranded RNA region is about identical in nucleotide sequence to the target gene ("sense region"), whereas the other strand is about identical in nucleotide sequence to the complement of the target gene or of the sense region ("antisense region"). It is expected that for silencing of the target gene expression, the nucleotide sequence of the 19 consecutive nucleotide sequences may have one mismatch, or the sense and antisense region may differ in one nucleotide. To achieve the construction of such RNA molecules or the encoding chimeric genes, use can be made of the vector as described in WO 02/059294.

Thus, in one aspect of the embodiment, the chimeric gene comprises the following operably linked DNA elements:

(a) a plant expressible promoter, preferably a plant expressible promoter which controls transcription preferentially in the fiber cells;
(b) a transcribed DNA region, which when transcribed yields a double-stranded RNA molecule capable of reducing the expression of the GLUC allele and the RNA molecule comprising a first and second RNA region wherein
    i) the first RNA region comprises a nucleotide sequence of at least 19 consecutive nucleotides having at least about 94% sequence identity to the nucleotide sequence of the GLUC allele;
    ii) the second RNA region comprises a nucleotide sequence complementary to the at least 19 consecutive nucleotides of the first RNA region;
    iii) the first and second RNA region are capable of base-pairing to form a double stranded RNA molecule between at least the 19 consecutive nucleotides of the first and second region; and
(c) a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of the plant.

The length of the first or second RNA region (sense or antisense region) may vary from about 19 nucleotides (nt) up to a length equaling the length (in nucleotides) of the GLUC allele. The total length of the sense or antisense nucleotide sequence may thus be at least about 25 nt, or at least about 50 nt, or at least about 100 nt, or at least about 150 nt, or at least about 200 nt, or at least about 500 nt. It is expected that there is no upper limit to the total length of the sense or the antisense nucleotide sequence. However for practical reasons (such as e.g. stability of the chimeric genes) it is expected that the length of the sense or antisense nucleotide sequence should not exceed 5000 nt, particularly should not exceed 2500 nt and could be limited to about 1000 nt.

It will be appreciated that the longer the total length of the sense or antisense region, the less stringent the requirements for sequence identity between these regions and the corresponding sequence in the GLUC allele or its complement. Preferably, the nucleic acid of interest should have a sequence identity of at least about 75% with the corresponding target sequence, particularly at least about 80%, more particularly at least about 85%, quite particularly about 90%, especially about 95%, more especially about 100%, quite especially be identical to the corresponding part of the target sequence or its complement. However, it is preferred that the nucleic acid of interest always includes a sequence of about 19 consecutive nucleotides, particularly about 25 nt, more particularly about 50 nt, especially about 100 nt, quite especially about 150 nt with 100% sequence identity to the corresponding part of the target nucleic acid. Preferably, for calculating the sequence identity and designing the corresponding sense or antisense sequence, the number of gaps should be minimized, particularly for the shorter sense sequences.

For the purpose of this invention, the "sequence identity" of two related nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (×100) divided by the number of positions compared. A gap, i.e., a position in an alignment where a residue is present in one sequence but not in the other, is regarded as a position with non-identical residues. The "optimal alignment" of two sequences is found by aligning the two sequences over the entire length according to the Needleman and Wunsch global alignment algorithm (Needleman and Wunsch, 1970, J Mol Biol 48(3):443-53) in The European Molecular Biology Open Software Suite (EMBOSS, Rice et al., 2000, Trends in Genetics 16(6): 276-277; see e.g. on the World Wide Web at ebi.ac.uk/emboss/align/index) using default settings (gap opening penalty=10 (for nucleotides)/10 (for proteins) and gap extension penalty=0.5 (for nucleotides)/0.5 (for proteins)). For nucleotides the default scoring matrix used is EDNAFULL and for proteins the default scoring matrix is EBLOSUM62.

"Substantially identical", "essentially similar", or "corresponding to", as used herein, refers to sequences, which, when optimally aligned as defined above, share at least a certain minimal percentage of sequence identity (as defined further below). "(A nucleotide or a nucleotide sequence) at a position corresponding to a position of (a nucleotide or a nucleotide sequence in a specific nucleotide sequence)", as used herein, refers to (nucleotides or nucleotide sequences) of two essentially similar sequences, which are aligned with each other in an optimal alignment of the two essentially similar sequences.

dsRNA encoding chimeric genes according to the invention may comprise an intron, such as a heterologous intron, located e.g. in the spacer sequence between the sense and antisense RNA regions in accordance with the disclosure of WO 99/53050 (incorporated herein by reference).

It is preferred for the current invention that the target specific gene sequence included in the antisense, sense or double stranded RNA molecule comprises at least one nucleotide, and preferably more which are specific for the specific GLUC allele whose expression is to be downregulated. Such specific nucleotides are indicated at least in FIG. **6** by the gray boxes.

In a preferred embodiment, the inhibitory RNA molecule is specifically adapted to downregulate the A-subgenomic allele of the GLUC1.1 gene. In another preferred embodiment, the biologically active RNA is specifically adapted to downregulate the D subgenome-specific allele of the GLUC1.1 gene.

The use of synthetic micro-RNA's to downregulate expression of a particular gene in a plant cell, provides for very high sequence specificity of the target gene, and thus allows conveniently to discriminate between closely related alleles as target genes the expression of which is to be downregulated.

Thus, in another embodiment of the invention, the inhibitory RNA or silencing RNA or biologically active RNA molecule may be a microRNA molecule, designed, synthesized and/or modulated to target and cause the cleavage of specific subgenomic alleles, preferably the A subgenomic allele of the GLUC1.1 gene in a fiber producing plant, such as a cotton plant. Various methods have been described to generate and use miRNAs for a specific target gene (including but not limited to Schwab et al. (2006, Plant Cell, 18(5):1121-1133), WO2006/044322, WO2005/047505, EP 06009836, incorporated by reference). Usually, an existing miRNA scaffold is modified in the target gene recognizing portion so that the generated miRNA now guides the RISC complex to cleave the RNA molecules transcribed from the target nucleic acid. miRNA scaffolds could be modified or synthesized such that the miRNA now comprises 21 consecutive nucleotides of one of the subgenomic alleles of the fiber selective β-1,3 endoglucanase encoding nucleotide sequence, such as the sequences represented in the Sequence listing of WO2008/083969, and allowing mismatches according to the herein below described rules.

Thus, in one embodiment, the invention provides a chimeric gene comprising the following operably linked DNA regions:

(a) a plant expressible promoter;
(b) a DNA region which upon introduction and transcription in a plant cell is processed into a miRNA, whereby the miRNA is capable of recognizing and guiding the cleavage of the mRNA of a GLUC allele of the plant but not another GLUC allele, such as the mRNA of the A subgenome specific GLUC allele but not the D subgenome specific GLUC allele; and optionally,
(c) a 3' DNA region involved in transcription termination and polyadenylation.

The mentioned DNA region processed into a miRNA may comprise a nucleotide sequence which is essentially complementary to a nucleotide sequence of at least 21 consecutive nucleotides of a GLUC allele, provided that one or more of following mismatches are allowed: a mismatch between the nucleotide at the 5' end of the miRNA and the corresponding nucleotide sequence in the RNA molecule; a mismatch between any one of the nucleotides in position 1 to position 9 of the miRNA and the corresponding nucleotide sequence in the RNA molecule; three mismatches between any one of the nucleotides in position 12 to position 21 of the miRNA and the corresponding nucleotide sequence in the RNA molecule provided that there are no more than two consecutive mismatches.

As used herein, a "miRNA" is an RNA molecule of about 20 to 22 nucleotides in length which can be loaded into a RISC complex and direct the cleavage of another RNA molecule, wherein the other RNA molecule comprises a nucleotide sequence essentially complementary to the nucleotide sequence of the miRNA molecule whereby one or more of the following mismatches may occur: a mismatch between the nucleotide at the 5' end of said miRNA and the corresponding nucleotide sequence in the target RNA molecule; a mismatch between any one of the nucleotides in position 1 to position 9 of said miRNA and the corresponding nucleotide sequence in the target RNA molecule; three mismatches between any one of the nucleotides in position 12 to position 21 of said miRNA and the corresponding nucleotide sequence in the target RNA molecule provided that there are no more than two consecutive mismatches. no mismatch is allowed at positions 10 and 11 of the miRNA (all miRNA positions are indicated starting from the 5' end of the miRNA molecule).

A miRNA is processed from a "pre-miRNA" molecule by proteins, such as DCL proteins, present in any plant cell and loaded onto a RISC complex where it can guide the cleavage of the target RNA molecules.

As used herein, a "pre-miRNA" molecule is an RNA molecule of about 100 to about 200 nucleotides, preferably about 100 to about 130 nucleotides which can adopt a secondary structure comprising a double stranded RNA stem and a single stranded RNA loop and further comprising the nucleotide sequence of the miRNA (and its complement sequence) in the double stranded RNA stem. Preferably, the miRNA and its complement are located about 10 to about 20 nucleotides from the free ends of the miRNA double stranded RNA stem. The length and sequence of the single stranded loop region are not critical and may vary considerably, e.g. between 30 and 50 nt in length. Preferably, the difference in free energy between unpaired and paired RNA structure is between −20 and −60 kcal/mole, particularly around −40 kcal/mole. The complementarity between the miRNA and the miRNA* need not be perfect and about 1 to 3 bulges of unpaired nucleotides can be tolerated. The secondary structure adopted by an RNA molecule can be predicted by computer algorithms conventional in the art such as mFOLD. The particular strand of the double stranded RNA stem from the pre-miRNA which is released by DCL activity and loaded onto the RISC complex is determined by the degree of complementarity at the 5' end, whereby the strand which at its 5' end is the least involved in hydrogen bounding between the nucleotides of the different strands of the cleaved dsRNA stem is loaded onto the RISC complex and will determine the sequence specificity of the target RNA molecule degradation. However, if empirically the miRNA molecule from a particular synthetic pre-miRNA molecule is not functional (because the "wrong" strand is loaded on the RISC complex, it will be immediately evident that this problem can be solved by exchanging the position of the miRNA molecule and its complement on the respective strands of the dsRNA stem of the pre-miRNA molecule. As is known in the art, binding between A and U involving two hydrogen bounds, or G and U involving two hydrogen bounds is less strong that between G and C involving three hydrogen bounds.

Naturally occurring miRNA molecules may be comprised within their naturally occurring pre-miRNA molecules but they can also be introduced into existing pre-miRNA molecule scaffolds by exchanging the nucleotide sequence of the miRNA molecule normally processed from such existing pre-miRNA molecule for the nucleotide sequence of another miRNA of interest. The scaffold of the pre-miRNA can also be completely synthetic. Likewise, synthetic miRNA molecules may be comprised within, and processed from, existing pre-miRNA molecule scaffolds or synthetic pre-miRNA scaffolds.

The pre-miRNA molecules (and consequently also the miRNA molecules) can be conveniently introduced into a plant cell by providing the plant cells with a gene comprising a plant-expressible promoter operably linked to a DNA region, which when transcribed yields the pre-miRNA molecule. The plant expressible promoter may be the promoter naturally associated with the pre-miRNA molecule or it may be a heterologous promoter.

Suitable miRNA and pre microRNA molecules for the specific downregulation of the expression of the GhGLUC1.1A gene are set forth in the sequence listing entries SEQ ID NO: 13, 14, 17, 18 and 19 of WO2008/083969.

Suitable miRNA and pre microRNA molecules for the specific downregulation of the expression of the GhGLUC1.1D gene are set forth in the sequence listing entries SEQ ID NO: 15, 16, 20 and 21 of WO2008/083969.

As used herein, the term "plant-expressible promoter" means a DNA sequence which is capable of controlling (initiating) transcription in a plant cell. This includes any promoter of plant origin, but also any promoter of non-plant origin which is capable of directing transcription in a plant cell, i.e., certain promoters of viral or bacterial origin such as the CaMV35S, the subterranean clover virus promoter No. 4 or No. 7, or T-DNA gene promoters and the like.

A plant-expressible promoter that controls initiation and maintenance of transcription preferentially in fiber cells is a promoter that drives transcription of the operably linked DNA region to a higher level in fiber cells and the underlying epidermis cells than in other cells or tissues of the plant. Such promoters include the promoter from cotton from a fiber-specific (3-tubulin gene (as described in WO0210377), the promoter from cotton from a fiber-specific actin gene (as described in WO0210413), the promoter from a fiber specific lipid transfer protein gene from cotton (as described in U.S. Pat. No. 5,792,933), a promoter from an expansin gene from cotton (WO9830698) or a promoter from a chitinase gene in cotton (US2003106097) or the promoters of the fiber specific genes described in U.S. Pat. No. 6,259,003 or U.S. Pat. No. 6,166,294. Fiber selective promoters as described herein may also be used.

The invention also encompasses the chimeric genes herein described, as well as plants, seeds, tissues comprising these chimeric genes, and fibers produced from such plants.

Methods to transform plants are well known in the art and are of minor relevance for the current invention. Methods to transform cotton plants are also well known in the art. *Agrobacterium*-mediated transformation of cotton has been described e.g. in U.S. Pat. No. 5,004,863 or in U.S. Pat. No. 6,483,013 and cotton transformation by particle bombardment is reported e.g. in WO 92/15675.

The chimeric genes according to the invention may be introduced into plants in a stable manner or in a transient manner using methods well known in the art. The chimeric genes may be introduced into plants, or may be generated inside the plant cell as described e.g. in EP 1339859.

The chimeric genes may be introduced by transformation in cotton plants from which embryogenic callus can be derived, such as Coker 312, Coker310, Coker 5Acala SJ-5, GSC25110, FIBERMAX 819, Siokra 1-3, T25, GSA75, Acala SJ2, Acala SJ4, Acala SJ5, Acala SJ-C1, Acala B1644, Acala B1654-26, Acala B1654-43, Acala B3991, Acala GC356, Acala GC510, Acala GAM1, Acala C1, Acala Royale, Acala Maxxa, Acala Prema, Acala B638, Acala B1810, Acala B2724, Acala B4894, Acala B5002, non Acala "picker" Siokra, "stripper" variety FC2017, Coker 315, STONEVILLE 506, STONEVILLE 825, DP50, DP61, DP90, DP77, DES119, McN235, HBX87, HBX191, HBX107, FC 3027, CHEMBRED A1, CHEMBRED A2, CHEMBRED A3, CHEMBRED A4, CHEMBRED B1, CHEMBRED B2, CHEMBRED B3, CHEMBRED C1, CHEMBRED C2, CHEMBRED C3, CHEMBRED C4, PAYMASTER 145, HS26, HS46, SICALA, PIMA S6 ORO BLANCO PIMA, FIBERMAX FM5013, FIBERMAX FM5015, FIBERMAX FM5017, FIBERMAX FM989, FIBERMAX FM832, FIBERMAX FM966, FIBERMAX FM958, FIBERMAX FM989, FIBERMAX FM958, FIBERMAX FM832, FIBERMAX FM991, FIBERMAX FM819, FIBERMAX FM800, FIBERMAX FM960, FIBERMAX FM966, FIBERMAX FM981, FIBERMAX FM5035, FIBERMAX FM5044, FIBERMAX FM5045, FIBERMAX FM5013, FIBERMAX FM5015, FIBERMAX FM5017 or FIBERMAX FM5024 and plants with genotypes derived thereof.

"Cotton" as used herein includes *Gossypium hirsutum, Gossypium barbadense, Gossypium arboreum* and *Gossypium herbaceum*. "Cotton progenitor plants" include *Gossypium arboreum, Gossypium herbaceum, Gossypium raimondii, Gossypium longicalyx* and *Gossypium kirkii.*

The methods and means of the current invention may also be employed for other plant species such as hemp, jute, flax and woody plants, including but not limited to *Pinus* spp., *Populus* spp., *Picea* spp., *Eucalyptus* spp. etc.

The obtained transformed plant can be used in a conventional breeding scheme to produce more transformed plants with the same characteristics or to introduce the chimeric gene according to the invention in other varieties of the same or related plant species, or in hybrid plants. Seeds obtained from the transformed plants contain the chimeric genes of the invention as a stable genomic insert and are also encompassed by the invention.

In one embodiment, the amount of functional GLUC protein is significantly reduced in fibers of the fiber-producing plant during the fiber strength building phase of fiber development compared to the amount of functional GLUC protein produced during the fiber strength building phase in a plant in which the functional expression of the at least one GLUC allele is not abolished.

A "significantly reduced amount of functional GLUC protein" (e.g. functional GLUC1.1A or GLUC1.1D protein) refers to a reduction in the amount of a functional GLUC protein produced by the cell comprising a mutant GLUC allele by at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 100% (i.e. no functional GLUC protein is produced by the cell) as compared to the amount of the functional GLUC protein produced by the cell not comprising the mutant GLUC allele. This definition encompasses the production of a "non-functional" GLUC protein (e.g. truncated GLUC protein) having no biological activity in vivo, the reduction in the absolute amount of the functional GLUC protein (e.g. no functional GLUC protein being made due to the mutation in the GLUC gene), and/or the production of a GLUC protein with significantly reduced biological activity compared to the activity of a functional wild type GLUC protein (such as a GLUC protein in which one or more amino acid residues that are crucial for the biological activity of the encoded GLUC protein, as exemplified above and below, are substituted for another amino acid residue). The term "mutant GLUC protein", as used herein, refers to a GLUC protein encoded by a mutant GLUC nucleic acid sequence ("gluc allele") whereby the mutation results in a significantly reduced and/or no GLUC activity in vivo, compared to the activity of the GLUC protein encoded by a non-mutant, wild type GLUC sequence ("GLUC allele").

In yet a further embodiment, the fibers of the non-naturally occurring fiber-producing plant have a higher callose content compared to the callose content of the fibers of an equivalent fiber-producing plant wherein the expression of the at least one GLUC allele is not abolished.

In a particular aspect of this embodiment, the strength of the fibers of the non-naturally occurring fiber-producing plant is increased compared to the strength of the fibers of an equivalent fiber-producing plant wherein the expression of the at least one GLUC allele is not abolished.

In one aspect of this embodiment, the non-naturally occurring *Gossypium* plant is a *Gossypium hirsutum* plant which is homozygous for the *Gossypium barbadense* GLUC1.1A allele. In a further aspect of this embodiment, the strength of the fibers of the *Gossypium* plant is on average between about 5% and about 10%, more specifically about 7.5%, higher than the fiber strength of a *Gossypium hirsutum* plant which is homozygous for the *Gossypium hirsutum* GLUC1.1A allele. In still a further aspect of this embodiment, the strength of the fibers of the *Gossypium* plant is on average between about 1.6 g/tex and about 3.3 g/tex, more specifically about 2.5 g/tex higher than the fiber strength of a *Gossypium hirsutum* plant which is homozygous for the *Gossypium hirsutum* GLUC1.1A allele. In yet a further aspect of this embodiment, the strength of the fibers of the *Gossypium* plant is on average between about 34.6 g/tex and about 36.3 g/tex, more specifically about 35.5 g/tex, as compared to a fiber strength of on average between about 32.2 g/tex and about 33.8 g/tex, more specifically about 33.0 g/tex of a *Gossypium hirsutum* plant which is homozygous for the *Gossypium hirsutum* GLUC1.1A allele.

Further provided herein are nucleic acid sequences of wild type and mutant GLUC1.1 genes/alleles from *Gossypium* species, as well as the wild type and mutant GLUC1.1 proteins. Also provided are methods of generating and combining mutant and wild type GLUC1.1 alleles in *Gossypium* plants, as well as *Gossypium* plants and plant parts comprising specific combinations of wild type and mutant GLUC1.1 alleles in their genome, whereby these plants produce fibers with altered fiber strength and whereby the plants preferably grow normally and have a normal phenotype. The use of these plants for transferring mutant GLUC1.1 alleles to other plants is also an embodiment of the invention, as are the plant products of any of the plants described. In addition kits and methods for marker assisted selection (MAS) for combining or detecting GLUC genes and/or alleles are provided. Each of the embodiments of the invention is described in detail herein below.

Provided are both wild type (GLUC1.1) nucleic acid sequences, encoding functional GLUC1.1 proteins, and mutant (gluc1.1) nucleic acid sequences (comprising one or more mutations, preferably mutations which result in a significantly reduced biological activity of the encoded GLUC1.1 protein or in no GLUC1.1 protein being produced) of GLUC1.1 genes from *Gossypium* species, especially from *Gossypium hirsutum* and *Gossypium barbadense*, but also from other *Gossypium* species. For example, *Gossypium* species comprising an A and/or a D genome may comprise different alleles of GLUC1.1A or GLUC1.1D genes which can be identified and combined in a single plant according to the invention. In addition, mutagenesis methods can be used to generate mutations in wild type GLUC1.1A or GLUC1.1D alleles, thereby generating mutant alleles for use according to the invention. Because specific GLUC1.1 alleles are preferably combined in a *Gossypium* plant by crossing and selection, in one embodiment the GLUC1.1 and/or gluc1.1 nucleic acid sequences are provided within a *Gossypium* plant (i.e. endogenously).

However, isolated GLUC1.1 and gluc1.1 nucleic acid sequences (e.g. isolated from the plant by cloning or made synthetically by DNA synthesis), as well as variants thereof and fragments of any of these are also provided herein, as these can be used to determine which sequence is present endogenously in a plant or plant part, whether the sequence encodes a functional protein or a protein with significantly reduced or no functionality (e.g. by expression in a recombinant host cell and enzyme assays) and for selection and transfer of specific alleles from one *Gossypium* plant into another, in order to generate a plant having the desired combination of functional and mutant alleles.

Nucleic acid sequences of GLUC1.1A and/or GLUC1.1D have been isolated from *Gossypium hirsutum*, from *Gossypium barbadense*, from *Gossypium tomentosum*, from *Gossypium darwinii*, from *Gossypium* mustelinum, from *Gossypium arboreum*, from *Gossypium herbaceum*, and from *Gossypium raimondii* as depicted in the sequence listing. The wild type GLUC1.1A sequences of *Gossypium hirsutum, tomentosum, mustelinum* and *herbaceum* and wild type GLUC1.1D sequences of *Gossypium hirsutum, tomentosum, barbadense, darwinii, mustelinum* and *raimondii* are depicted, while the mutant gluc1.1a and/or gluc1.1d sequences of these sequences, and of sequences essentially similar to these, are described herein below and in the Examples, with reference to the wild type GLUC1.1A and GLUC1.1D sequences. Further, the mutant GLUC1.1A sequences of *Gossypium barbadense, darwinii* and *arboreum* are depicted, while the alternative mutant gluc1.1a sequences of these sequences, and of sequences essentially similar to these, are described herein below and in the Examples. The genomic GLUC1.1A and D protein-encoding DNA, and corresponding pre-mRNA, comprises 2 exons (numbered exons 1 and 2 starting from the 5' end) interrupted by 1 intron. In the cDNA and corresponding processed mRNA (i.e. the spliced RNA), introns are removed and exons are joined, as depicted in the sequence listing and FIGS. **1** and **6**. Exon sequences are more conserved evolutionarily and are therefore less variable than intron sequences.

"GLUC1.1A nucleic acid sequences" or "GLUC1.1A variant nucleic acid sequences" according to the invention are nucleic acid sequences encoding an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 4 or nucleic acid sequences encoding a cDNA sequence with at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 3 or comprises a coding sequence with at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the nucleotide at position 2410 to the nucleotide at position 3499 of SEQ ID NO: 1. These nucleic acid sequences may also be referred to as being "essentially similar" or "essentially identical" or "corresponding to" the GLUC1.1A sequences provided in the sequence listing.

"GLUC1.1D nucleic acid sequences" or "GLUC1.1D variant nucleic acid sequences" according to the invention are nucleic acid sequences encoding an amino acid sequence having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% sequence identity to SEQ ID NO: 10 or nucleic acid sequences encoding a cDNA sequence with at least 97%, at least 98%, at least 99% sequence identity to SEQ ID NO: 3 or comprises a coding sequence with at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the nucleotide at position 3337 to the nucleotide at position 4444 of SEQ ID NO: 7. These nucleic acid sequences may also be referred to as being "essentially similar" or "essentially identical" or "corresponding to" the GLUC1.1A sequences provided in the sequence listing.

Thus, the invention provides both nucleic acid sequences encoding wild type, functional GLUC1.1A and GLUC1.1D proteins, including variants and fragments thereof (as defined further below), as well as mutant nucleic acid sequences of any of these, whereby the mutation in the nucleic acid sequence preferably results in one or more amino acids being inserted, deleted or substituted in comparison to the wild type protein. Preferably the mutation(s) in the nucleic acid sequence result in one or more amino acid changes (i.e. in relation to the wild type amino acid sequence one or more amino acids are inserted, deleted and/or substituted) whereby the biological activity of the GLUC1.1 protein is significantly reduced. A significant reduction in biological activity of the mutant GLUC1.1 protein, refers to a reduction in enzymatic activity by at least 30%, at least 40%, 50% or more, at least 90% or 100% (no biological activity) compared to the activity of the wild type protein.

Both endogenous and isolated nucleic acid sequences are provided herein. Also provided are fragments of the GLUC1.1 sequences and GLUC1.1 variant nucleic acid sequences defined above, for use as primers or probes and as components of kits according to another aspect of the invention (see further below). A "fragment" of a GLUC1.1 or gluc1.1 nucleic acid sequence or variant thereof (as defined) may be of various lengths, such as at least 10, 12, 15, 18, 20, 50, 100, 200, 500, 1000 contiguous nucleotides of the GLUC1.1 or gluc1.1 sequence (or of the variant sequence).

Nucleic acid sequences of GLUC1.1A and/or GLUC1.1D have been isolated from *Gossypium hirsutum*, from *Gossypium barbadense*, from *Gossypium tomentosum*, from *Gossypium darwinii*, from *Gossypium mustelinum*, from *Gossypium arboreum*, from *Gossypium herbaceum*, and from *Gossypium raimondii* as depicted in the sequence listing. The wild type GLUC1.1A sequences of *Gossypium hirsutum, tomentosum, mustelinum* and *herbaceum* and wild type GLUC1.1D sequences of *Gossypium hirsutum, tomentosum, barbadense, darwinii, mustelinum* and *raimondii* are depicted, while the mutant gluc1.1a and/or gluc1.1d sequences of these sequences, and of sequences essentially similar to these, are described herein below and in the Examples, with reference to the wild type GLUC1.1A and GLUC1.1D sequences. Further, the mutant GLUC1.1A sequences of *Gossypium barbadense, darwinii* and *arboreum* are depicted, while the alternative mutant gluc1.1a sequences of these sequences, and of sequences essentially similar to these, are described herein below and in the Examples. The genomic GLUC1.1A and D protein-encoding DNA, and corresponding pre-mRNA, comprises 2 exons (numbered exons 1 and 2 starting from the 5' end) interrupted by 1 intron. In the cDNA and corresponding processed mRNA (i.e. the spliced RNA), introns are removed and exons are joined, as depicted in the sequence listing and FIGS. **1** and **6**. Exon sequences are more conserved evolutionarily and are therefore less variable than intron sequences.

The nucleic acid sequences of GLUC1.1A and/or GLUC1.1D from *Gossypium hirsutum*, from *Gossypium barbadense*, from *Gossypium tomentosum*, from *Gossypium darwinii*, from *Gossypium mustelinum*, from *Gossypium arboreum*, from *Gossypium herbaceum*, and from *Gossypium raimondii* depicted in the sequence listing encode wild type, functional GLUC1.1 proteins from these *Gossypium* species. Further, the mutant GLUC1.1A sequences of *Gossypium barbadense, darwinii* and *arboreum* depicted in the sequence listing encode wild type, non-functional GLUC1.1 proteins from these *Gossypium* species. Thus, these sequences are endogenous to the *Gossypium* species from which they were isolated. Other *Gossypium* species, varieties, breeding lines or wild accessions may be screened for other GLUC1.1A and GLUC1.1D alleles, encoding the same GLUC1.1A and GLUC1.1D proteins or variants thereof. For example, nucleic acid hybridization techniques (e.g. Southern blot, using for example stringent hybridization conditions) or PCR-based techniques may be used to identify GLUC1.1 alleles endogenous to other *Gossypium* plants. To screen such plants or plant tissues for the presence of GLUC1.1 alleles, the GLUC1.1 nucleic acid sequences provided in the sequence listing, or variants or fragments of any of these, may be used. For example whole sequences or fragments may be used as probes or primers. For example specific or degenerate primers may be used to amplify nucleic acid sequences encoding GLUC1.1 proteins from the genomic DNA of the plant or plant tissue. These GLUC1.1 nucleic acid sequences may be isolated and sequenced using standard molecular biology techniques. Bioinformatics analysis may then be used to characterize the allele(s), for example in order to determine which GLUC1.1 allele the sequence corresponds to and which GLUC1.1 protein or protein variant is encoded by the sequence.

Whether a nucleic acid sequence encodes a functional GLUC1.1 protein can be analyzed by recombinant DNA techniques as known in the art, e.g. expressing the nucleic acid molecule in a host cell (e.g. a bacterium, such as *E. coli*) and analyzing the endo-1,3-beta-glucanase activity of the resulting protein or cells.

In addition, it is understood that GLUC1.1 nucleic acid sequences and variants thereof (or fragments of any of these) may be identified in silico, by screening nucleic acid databases for essentially similar sequences. Likewise, a nucleic acid sequence may be synthesized chemically. Fragments of nucleic acid molecules according to the invention are also provided, which are described further below. Fragments include nucleic acid sequences encoding only the mature protein, or smaller fragments comprising all or part of the exon and/or intron sequences, etc.

Nucleic acid sequences comprising one or more nucleotide deletions, insertions or substitutions relative to the wild type nucleic acid sequences are another embodiment of the invention, as are fragments of such mutant nucleic acid molecules. Such mutant nucleic acid sequences (referred to as gluc1.1 sequences) can be generated and/or identified using various known methods, as described further below. Again, such nucleic acid molecules are provided both in endogenous form and in isolated form. In one embodiment, the mutation(s) result in one or more changes (deletions, insertions and/or substitutions) in the amino acid sequence of the encoded GLUC1.1 protein (i.e. it is not a "silent mutation"). In another embodiment, the mutation(s) in the nucleic acid sequence result in a significantly reduced or completely abolished biological activity of the encoded GLUC1.1 protein relative to the wild type protein.

The nucleic acid molecules may, thus, comprise one or more mutations, such as:

(a) a "missense mutation", which is a change in the nucleic acid sequence that results in the substitution of an amino acid for another amino acid;
(b) a "nonsense mutation" or "STOP codon mutation", which is a change in the nucleic acid sequence that results in the introduction of a premature STOP codon and thus the termination of translation (resulting in a truncated protein); plant genes contain the translation stop codons "TGA" (UGA in RNA), "TAA" (UAA in RNA) and "TAG" (UAG in RNA); thus any nucleotide substitution, insertion, deletion which results in one of these codons to be in the mature mRNA being translated (in the reading frame) will terminate translation.
(c) an "insertion mutation" of one or more amino acids, due to one or more codons having been added in the coding sequence of the nucleic acid;
(d) a "deletion mutation" of one or more amino acids, due to one or more codons having been deleted in the coding sequence of the nucleic acid;
(e) a "frameshift mutation", resulting in the nucleic acid sequence being translated in a different frame downstream of the mutation. A frameshift mutation can have various causes, such as the insertion, deletion or duplication of one or more nucleotides, but also mutations which affect pre-mRNA splicing (splice site mutations) can result in frameshifts;
(f) a "splice site mutation", which alters or abolishes the correct splicing of the pre-mRNA sequence, resulting in a protein of different amino acid sequence than the wild type. For example, one or more exons may be skipped during RNA splicing, resulting in a protein lacking the amino acids encoded by the skipped exons. Alternatively, the reading frame may be altered through incorrect splicing, or one or more introns may be retained, or alternate splice donors or acceptors may be generated, or splicing may be initiated at an alternate position (e.g. within an intron), or alternate polyadenylation signals may be generated. Correct pre-mRNA splicing is a complex process, which can be affected by various mutations in the nucleotide sequence of the GLUC1.1-encoding gene. In higher eukaryotes, such as plants, the major spliceosome splices introns containing GU at the 5' splice site (donor site) and AG at the 3' splice site (acceptor site). This GU-AG rule (or GT-AG rule; see Lewin, Genes VI, Oxford University Press 1998, pp 885-920, ISBN 0198577788) is followed in about 99% of splice sites of nuclear eukaryotic genes, while introns containing other dinucleotides at the 5' and 3' splice site, such as GC-AG and AU-AC account for only about 1% and 0.1% respectively.

As already mentioned, it is desired that the mutation(s) in the nucleic acid sequence preferably result in a mutant protein comprising significantly reduced or no enzymatic activity in vivo. Basically, any mutation which results in a protein comprising at least one amino acid insertion, deletion and/or substitution relative to the wild type protein can lead to significantly reduced or no enzymatic activity. It is, however, understood that mutations in certain parts of the protein are more likely to result in a reduced function of the mutant GLUC1.1 protein, such as mutations leading to truncated proteins, whereby significant portions of the functional domains, such as the catalytic domain, are lacking.

The functional GLUC1.1 proteins of *Gossypium* described herein are about 325-337 amino acids in length and comprise a number of structural and functional domains. These include the following: An N-terminal plastid target peptide of about 14-26 amino acids followed by what constitutes the mature GLUC1.1 protein. The mature GLUC1.1 protein comprises active site and glycosylation amino acid residues as indicated in Table 4 above.

Thus in one embodiment, nucleic acid sequences comprising one or more of any of the types of mutations described above are provided. In another embodiment, gluc1.1 sequences comprising one or more deletion mutations, one or more stop codon (nonsense) mutations and/or one or more splice site mutations are provided. Any of the above mutant nucleic acid sequences are provided per se (in isolated form), as are plants and plant parts comprising such sequences endogenously.

A deletion mutation in a GLUC1.1 allele, as used herein, is a mutation in a GLUC1.1 allele whereby at least 1, at least 2, 3, 4, 5, 10, 20, 30, 50, 100, 200, 500, 1000 or more bases are deleted from the corresponding wild type GLUC1.1 allele, and whereby the deletion results in the mutant GLUC1.1 allele being transcribed and translated into a mutant protein which has significantly reduced or no activity in vivo. A deletion may lead to a frame-shift and/or it may introduce a premature stop codon, or may lead to one amino acid or more amino acids (e.g. large parts) of coding sequence being removed, etc. The exact underlying molecular basis by which the deletion results in a mutant protein having significantly reduced biological activity is not important. Also provided herein are plants and plant parts in which specific GLUC1.1 alleles are completely deleted, i.e. plants and plant parts lacking one or more GLUC1.1 alleles.

A nonsense mutation in a GLUC1.1 allele, as used herein, is a mutation in a GLUC1.1 allele whereby one or more translation stop codons are introduced into the coding DNA and the corresponding mRNA sequence of the corresponding wild type GLUC1.1 allele. Translation stop codons are TGA (UGA in the mRNA), TAA (UAA) and TAG (UAG). Thus, any mutation (deletion, insertion or substitution) which leads to the generation of an in-frame stop codon in the coding sequence (exon sequence) will result in termination of translation and truncation of the amino acid chain. In one embodiment, a mutant GLUC1.1 allele comprising a nonsense mutation is a GLUC1.1 allele wherein an in-frame stop codon is introduced in the GLUC1.1 codon sequence by a single nucleotide substitution, such as the mutation of CAG to TAG, TGG to TAG, TGG to TGA, or CGA to TGA. In another embodiment, a mutant GLUC1.1 allele comprising a nonsense mutation is a GLUC1.1 allele wherein an in-frame stop codon is introduced in the GLUC1.1 codon sequence by double nucleotide substitutions, such as the mutation of CAG to TAA, TGG to TAA, CGG to TAG or TGA, CGA to TAA. In yet another embodiment, a mutant GLUC1.1 allele comprising a nonsense mutation is a GLUC1.1 allele wherein an in-frame stop codon is introduced in the GLUC1.1 codon sequence by triple nucleotide substitutions, such as the mutation of CGG to TAA. The truncated protein lacks the amino acids encoded by the coding DNA downstream of the mutation (i.e. the C-terminal part of the GLUC1.1 protein) and maintains the amino acids encoded by the coding DNA upstream of the mutation (i.e. the N-terminal part of the GLUC1.1 protein). In one embodiment, the nonsense mutation is present anywhere in front of the second conserved Glu residue, the Trp residue, the first Glu residue, and/or the Tyr residue of the active site, so that at least the conserved Glu residue, the Trp residue, the first Glu residue, and/or the Tyr residue is lacking, resulting in significantly reduced activity of the truncated protein. The more truncated the mutant protein is in comparison to the wild type protein, the more likely it is that it will lack any enzymatic activity. Thus in another embodiment, a mutant GLUC1.1 allele comprising a nonsense mutation which result in a truncated protein lacking the second conserved Glu, a truncated protein lacking the second conserved Glu residue and the Trp residue, a truncated protein lacking the second conserved Glu residue, the Trp residue and the first Glu residue, a truncated protein lacking the second conserved Glu residue, the Trp residue, the first Glu residue and the Tyr residue, or a truncated protein with even less amino acids in length are provided. In yet another embodiment, the nonsense mutation results in one or more exons not being translated into protein, such as exon 1, exon 2 or exons 1 and 2.

A splice site mutation in a GLUC1.1 allele, as used herein, is a mutation in a GLUC1.1 allele whereby a mutation in the corresponding wild type functional GLUC1.1 allele results in aberrant splicing of the pre-mRNA thereby resulting in a mutant protein having significantly reduced or no activity. The mutation may be in the consensus splice site sequence. For example, Table 5 describes consensus sequences, which—if mutated—are likely to affect correct splicing. The GT-AG splice sites commonly have other conserved nucleotides, such as 2 highly conserved nucleotides on the 5' end of the intron (in the exon), often being 5'-AG-3'. On the 3'-side of the GT dinucleotide (thus in the intron) high conservation can be found for a tetranucleotide 5'-AAGT-3'. This means that 8 nucleotides can be identified as highly conserved at the donor site.

TABLE 5

Consensus splice site sequences

| Intron type | 5' splice junction (exon^intron) | Near 3'splice site | 3'splice junction (intron^exon) | Found in |
|---|---|---|---|---|
| GU-AG (Canonical introns; about 99%) | CRN^**GU**(A/G)AGU | A | Yn**AG**^N | nuclear pre-mRNA |
| (about 1%) | ^**GC** | | **AG**^ | nuclear pre-mRNA |
| Non-canonical introns (< about 0.1%) | ^**AU** | | **AC**^ | nuclear pre-mRNA |
| Canonical branch sites | | CUPuAPy | | 20-50 nucleotides 5' to splice-site acceptor of nuclear pre mRNA |

^depicts the splice site; R = A or G; Y = C or T; N = A, C, G or T (but often G); n = multiple nucleotides; in bold = consensus dinucleotides in the intron sequence.
Pu = purine base; Py = pyrimidine base.

Splice site structure and consensus sequences are described in the art and computer programs for identifying exons and splice site sequences, such as NetPLAntgene, BDGP or Genio, est2genome, FgeneSH, and the like, are available. Comparison of the genomic sequence or pre-mRNA sequence with the translated protein can be used to determine or verify splice sites and aberrant splicing.

Any mutation (insertion, deletion and/or substitution of one or more nucleotides) which alters pre-mRNA splicing and thereby leads to a protein with significantly reduced biological activity is encompassed herein. In one embodiment, a mutant GLUC1.1 allele comprising a splice site mutation is a GLUC1.1 allele wherein altered splicing is caused by the introduction in the GLUC1.1 transcribed DNA region of one or more nucleotide substitution(s) of the consensus dinucleotides depicted in bold above. For example, ˆGU may for example be mutated to ˆAU in the donor splice site and/or AGˆ may be mutated to AAˆ in the acceptor splice site sequence. In another embodiment, a mutant GLUC1.1 allele comprising a splice site mutation is a GLUC1.1 allele wherein altered splicing is caused by the introduction in the GLUC1.1 transcribed DNA region of one or more nucleotide substitution(s) in the conserved nucleotides in the exon sequences.

Further provided are both functional GLUC1.1 amino acid sequences and non-functional GLUC1.1 amino acid sequences (comprising one or more mutations, preferably mutations which result in a significantly reduced or no biological activity of the GLUC1.1 protein) from *Gossypium* species, especially from *Gossypium hirsutum* and *Gossypium barbadense*, but also from other *Gossypium* species, such as those indicated below. In addition, mutagenesis methods can be used to generate mutations in wild type functional GLUC1.1 alleles, thereby generating mutant non-functional GLUC1.1 alleles which can encode further non-functional GLUC1.1 proteins. In one embodiment the functional and/or non-functional GLUC1.1 amino acid sequences are provided within a *Gossypium* plant (i.e. endogenously). However, isolated GLUC1.1 amino acid sequences (e.g. isolated from the plant or made synthetically), as well as variants thereof and fragments of any of these are also provided herein.

Amino acid sequences of GLUC1.1A and GLUC1.1D proteins have been determined from *Gossypium hirsutum*, from *Gossypium barbadense*, from *Gossypium* tomentosum, from *Gossypium darwinii*, from *Gossypium mustilinum*, from *Gossypium arboreum*, from *Gossypium herbaceum*, and from *Gossypium raimondii* as depicted in the sequence listing and FIGS. **2** and **7**. The wild type functional GLUC1.1A sequences of *Gossypium hirsutum, tomentosum, mustilinum* and *herbaceum* and wild type functional GLUC1.1D sequences of *Gossypium hirsutum, tomentosum, barbadense, darwinii, mustilinum* and *raimondii* are depicted, while mutant non-functional GLUC1.1A sequences of these, and of sequences essentially similar to these, are described herein below, with reference to the wild type functional GLUC1.1A and GLUC1.1D sequences. Further, the wild type non-functional GLUC1.1A sequences of *Gossypium barbadense, darwinii* and *arboreum* are depicted, while alternative (mutant) non-functional GLUC1.1A sequences of these sequences, and of sequences essentially similar to these, are described herein below and in the Examples.

As described above, the functional GLUC1.1 proteins of *Gossypium* described herein are about 325-337 amino acids in length and comprise a number of structural and functional domains. The sequences of the N-terminal part of the GLUC1.1 proteins are less conserved evolutionarily than the sequences of the mature GLUC1.1 proteins. The sequences of the mature GLUC1.1 proteins are therefore less variable than the sequences of the precursor proteins.

"GLUC1.1A amino acid sequences" or "GLUC1.1A variant amino acid sequences" according to the invention are amino acid sequences having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or at least 100% sequence identity to SEQ ID NO: 4. These amino acid sequences may also be referred to as being "essentially similar" or "essentially identical" or "corresponding to" the GLUC1.1A sequences provided in the sequence listing.

"GLUC1.1D amino acid sequences" or "GLUC1.1D variant amino acid sequences" according to the invention are amino acid sequences having at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or at least 100% sequence identity to SEQ ID NO: 10. These amino acid sequences may also be referred to as being "essentially similar" or "essentially identical" or "corresponding to" the GLUC1.1D sequences provided in the sequence listing.

Thus, the invention provides both amino acid sequences of wild type functional and non-functional GLUC1.1A and GLUC1.1D proteins, including variants and fragments thereof (as defined further below), as well as mutant non-functional amino acid sequences of any of these, whereby the mutation in the amino acid sequence preferably results in a significant reduction in the biological activity of the GLUC1.1 protein. A significant reduction in biological activity of the (wild type or mutant) non-functional GLUC1.1 protein, refers to a reduction in enzymatic activity (i.e. in endo-1,3-beta-glucanase activity) by at least 30%, at least 40%, 50% or more, at least 90% or 100% (no biological activity) compared to the activity of the functional protein.

Both endogenous and isolated amino acid sequences are provided herein. A "fragment" of a GLUC1.1 amino acid sequence or variant thereof (as defined) may be of various lengths, such as at least 10, 12, 15, 18, 20, 50, 100, 200, 400 contiguous amino acids of the GLUC1.1 sequence (or of the variant sequence).

The amino acid sequences depicted in the sequence listing are wild type GLUC1.1 proteins from *Gossypium* species. Thus, these sequences are endogenous to the *Gossypium* plants from which they were isolated. Other *Gossypium* species, varieties, breeding lines or wild accessions may be screened for other (functional or non-functional) GLUC1.1 proteins with the same amino acid sequences or variants thereof, as described above.

In addition, it is understood that GLUC1.1 amino acid sequences and variants thereof (or fragments of any of these) may be identified in silico, by screening amino acid databases for essentially similar sequences. Fragments of amino acid molecules according to the invention are also provided. Fragments include amino acid sequences of the mature protein, or smaller fragments comprising all or part of the amino acid sequences, etc.

Amino acid sequences comprising one or more amino acid deletions, insertions or substitutions relative to the wild type (functional or non-functional) amino acid sequences are another embodiment of the invention, as are fragments of such mutant amino acid molecules. Such mutant amino acid sequences can be generated and/or identified using various known methods, as described above. Again, such amino acid molecules are provided both in endogenous form and in isolated form.

In one embodiment, the mutation(s) in the amino acid sequence result in a significantly reduced or completely abolished biological activity of the GLUC1.1 protein relative to the wild type protein. As described above, basically, any mutation which results in a protein comprising at least one amino acid insertion, deletion and/or substitution relative to the wild type protein can lead to significantly reduced (or no) enzymatic activity. It is, however, understood that mutations in certain parts of the protein are more likely to result in a reduced function of the mutant GLUC1.1 protein, such as mutations leading to truncated proteins, whereby significant portions of the functional domains, such as the active site or glycosylation site (see above), are lacking or mutations whereby conserved amino acid residues which have a catalytic function or which are involved in substrate specificity are substituted.

Thus in one embodiment, mutant GLUC1.1 proteins are provided comprising one or more deletion or insertion mutations, whereby the deletion(s) or insertion(s) result(s) in a mutant protein which has significantly reduced or no activity in vivo. Such mutant GLUC1.1 proteins are GLUC1.1 proteins wherein at least 1, at least 2, 3, 4, 5, 10, 20, 30, 50, 100, 200, 300, 400 or more amino acids are deleted or inserted as compared to the wild type GLUC1.1 protein, whereby the deletion(s) or insertion(s) result(s) in a mutant protein which has significantly reduced or no activity in vivo.

In another embodiment, mutant GLUC1.1 proteins are provided which are truncated whereby the truncation results in a mutant protein which has significantly reduced or no activity in vivo. Such truncated GLUC1.1 proteins are GLUC1.1 proteins which lack functional domains, such as active site residues and/or glycosylation site residues, in the C-terminal part of the corresponding wild type (mature) GLUC1.1 protein and which maintain the N-terminal part of the corresponding wild type (mature) GLUC1.1 protein. Thus in one embodiment, a truncated GLUC1.1 protein comprising the N-terminal part of the corresponding wild type (mature) GLUC1.1 protein up to but not including the conserved second Glu residue (as described above) is provided. The more truncated the mutant protein is in comparison to the wild type protein, the more likely it is that it will lack any enzymatic activity. Thus in another embodiment, a truncated GLUC1.1 protein comprising the N-terminal part of the corresponding wild type (mature) GLUC1.1 protein up to but not including the conserved Trp and/or the first Glu residue (as described above) is provided. In yet another embodiment, a truncated GLUC1.1 protein comprising the N-terminal part of the corresponding wild type (mature) GLUC1.1 protein up to but not including the conserved Tyr residue (as described above), or lacking even more amino acids, is provided.

In yet another embodiment, mutant GLUC1.1 proteins are provided comprising one or more substitution mutations, whereby the substitution(s) result(s) in a mutant protein which has significantly reduced or no activity in vivo. Such mutant GLUC1.1 proteins are GLUC1.1 proteins whereby conserved amino acid residues which have a catalytic function or which are involved in substrate binding or specificity (for example, those described above) are substituted. Thus in one embodiment, a mutant GLUC1.1 protein comprising a substitution of a conserved amino acid residue which has a catalytic function, such as the conserved first or second Glu, Trp, and/or Tyr residues, is provided. In another embodiment, a mutant GLUC1.1 protein comprising a substitution of a conserved amino acid residue involved in glycosylation, such as the conserved Asn residue, is provided.

In another aspect of the invention, methods are provided for generating mutant gluc1.1 alleles (for example induced by mutagenesis) and/or identifying mutant gluc1.1 alleles using a range of methods, which are conventional in the art, for example using PCR based methods to amplify part or all of the gluc1.1 genomic or cDNA.

The term "mutagenesis", as used herein, refers to the process in which plant cells (e.g., a plurality of *Gossypium* seeds or other parts, such as pollen) are subjected to a technique which induces mutations in the DNA of the cells, such as contact with a mutagenic agent, such as a chemical substance (such as ethylmethylsulfonate (EMS), ethylnitrosourea (ENU), etc.) or ionizing radiation (neutrons (such as in fast neutron mutagenesis, etc.), alpha rays, gamma rays (such as that supplied by a Cobalt 60 source), X-rays, UV-radiation, etc.), or a combination of two or more of these. Thus, the desired mutagenesis of one or more GLUC1.1 alleles may be accomplished by use of chemical means such as by contact of one or more plant tissues with ethylmethylsulfonate (EMS), ethylnitrosourea, etc., by the use of physical means such as x-ray, etc, or by gamma radiation, such as that supplied by a Cobalt 60 source.

Following mutagenesis, *Gossypium* plants are grown from the treated seeds, or regenerated from the treated cells using known techniques. For instance, the resulting *Gossypium* seeds may be planted in accordance with conventional growing procedures and following self-pollination seed is formed on the plants. Additional seed which is formed as a result of such self-pollination in the present or a subsequent generation may be harvested and screened for the presence of mutant GLUC1.1 alleles, using techniques which are conventional in the art, for example polymerase chain reaction (PCR) based techniques (amplification of the gluc1.1 alleles) or hybridization based techniques, e.g. Southern blot analysis, and/or direct sequencing of gluc1.1 alleles. To screen for the presence of point mutations (so called Single Nucleotide Polymorphisms or SNPs) in mutant GLUC1.1 alleles, SNP detection methods conventional in the art can be used, for example oligoligation-based techniques, single base extension-based techniques or techniques based on differences in restriction sites, such as TILLING.

As described above, mutagenization (spontaneous as well as induced) of a specific wild-type (functional or non-functional) GLUC1.1 allele results in the presence of one or more deleted, inserted, or substituted nucleotides (hereinafter called "mutation region") in the resulting mutant GLUC1.1 allele. The mutant GLUC1.1 allele can thus be characterized by the location and the configuration of the one or more deleted, inserted, or substituted nucleotides in the wild type GLUC1.1 allele. The site in the wild type GLUC1.1 allele where the one or more nucleotides have been inserted, deleted, or substituted, respectively, is also referred to as the "mutation region". A "5' or 3' flanking region or sequence" as used herein refers to a DNA region or sequence in the mutant (or the corresponding wild type) GLUC1.1 allele of at least 20 bp, preferably at least 50 bp, at least 750 bp, at least 1500 bp, and up to 5000 bp of DNA different from the DNA containing the one or more deleted, inserted, or substituted nucleotides, preferably DNA from the mutant (or the corresponding wild type) GLUC1.1 allele which is located either immediately upstream of and contiguous with (5' flanking region or sequence") or immediately downstream of and contiguous with (3' flanking region or sequence") the mutation region in the mutant GLUC1.1 allele (or in the corresponding wild type GLUC1.1 allele).

The tools developed to identify a specific mutant GLUC1.1 allele or the plant or plant material comprising a specific mutant GLUC1.1 allele, or products which comprise plant material comprising a specific mutant GLUC1.1 allele are based on the specific genomic characteristics of the specific mutant GLUC1.1 allele as compared to the genomic characteristics of the corresponding wild type GLUC1.1 allele, such as, a specific restriction map of the genomic region comprising the mutation region, molecular markers or the sequence of the flanking and/or mutation regions.

Once a specific mutant GLUC1.1 allele has been sequenced, primers and probes can be developed which specifically recognize a sequence within the 5' flanking, 3' flanking and/or mutation regions of the mutant GLUC1.1 allele in the nucleic acid (DNA or RNA) of a sample by way of a molecular biological technique. For instance a PCR method can be developed to identify the mutant GLUC1.1 allele in biological samples (such as samples of plants, plant material or products comprising plant material). Such a PCR is based on at least two specific "primers": one recognizing a sequence within the 5' or 3' flanking region of the mutant GLUC1.1 allele and the other recognizing a sequence within the 3' or 5' flanking region of the mutant GLUC1.1 allele, respectively; or one recognizing a sequence within the 5' or 3' flanking region of the mutant GLUC1.1 allele and the other recognizing a sequence within the mutation region of the mutant GLUC1.1 allele; or one recognizing a sequence within the 5' or 3' flanking region of the mutant GLUC1.1 allele and the other recognizing a sequence spanning the joining region between the 3' or 5' flanking region and the mutation region of the specific mutant GLUC1.1 allele (as described further below), respectively.

The primers preferably have a sequence of between 15 and 35 nucleotides which under optimized PCR conditions "specifically recognize" a sequence within the 5' or 3' flanking region, a sequence within the mutation region, or a sequence spanning the joining region between the 3' or 5' flanking and mutation regions of the specific mutant GLUC1.1 allele, so that a specific fragment ("mutant GLUC1.1 specific fragment" or discriminating amplicon) is amplified from a nucleic acid sample comprising the specific mutant GLUC1.1 allele. This means that only the targeted mutant GLUC1.1 allele, and no other sequence in the plant genome, is amplified under optimized PCR conditions.

PCR primers suitable for the invention may be the following:

- oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides selected from the 5' flanking sequence of a specific mutant GLUC1.1 allele (i.e., for example, the sequence 5' flanking the one or more nucleotides deleted, inserted or substituted in the mutant GLUC1.1 alleles of the invention, such as the sequence 5' flanking the deletion, non-sense or splice site mutations described above or the sequence 5' flanking the potential STOP codon or splice site mutations indicated above) at their 3' end (primers recognizing 5' flanking sequences); or
- oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the 3' flanking sequence of a specific mutant GLUC1.1 allele (i.e., for example, the complement of the sequence 3' flanking the one or more nucleotides deleted, inserted or substituted in the mutant GLUC1.1 alleles of the invention, such as the complement of the sequence 3' flanking the deletion, non-sense or splice site mutations described above or the complement of the sequence 3' flanking the potential STOP codon or splice site mutations indicated above) at their 3' end (primers recognizing 3' flanking sequences); or
- oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 nucleotides selected from the sequence of the mutation region of a specific mutant GLUC1.1 allele (i.e., for example, the sequence of nucleotides inserted or substituted in the GLUC1.1 genes of the invention, or the complement thereof) at their 3' end (primers recognizing mutation sequences).

The primers may of course be longer than the mentioned 17 consecutive nucleotides, and may e.g. be 20, 21, 30, 35, 50, 75, 100, 150, 200 nt long or even longer. The primers may entirely consist of nucleotide sequence selected from the mentioned nucleotide sequences of flanking and mutation sequences. However, the nucleotide sequence of the primers at their 5' end (i.e. outside of the 3'-located 17 consecutive nucleotides) is less critical. Thus, the 5' sequence of the primers may consist of a nucleotide sequence selected from the flanking or mutation sequences, as appropriate, but may contain several (e.g. 1, 2, 5, 10) mismatches. The 5' sequence of the primers may even entirely consist of a nucleotide sequence unrelated to the flanking or mutation sequences, such as e.g. a nucleotide sequence representing restriction enzyme recognition sites. Such unrelated sequences or flanking DNA sequences with mismatches should preferably be not longer than 100, more preferably not longer than 50 or even 25 nucleotides.

Moreover, suitable primers may comprise or consist of a nucleotide sequence at their 3' end spanning the joining region between flanking and mutation sequences (i.e., for example, the joining region between a sequence 5' flanking one or more nucleotides deleted, inserted or substituted in the mutant GLUC1.1 alleles of the invention and the sequence of the one or more nucleotides inserted or substituted or the sequence 3' flanking the one or more nucleotides deleted, such as the joining region between a sequence 5' flanking deletion, non-sense or splice site mutations in the GLUC1.1 genes of the invention described above and the sequence of the non-sense or splice site mutations or the sequence 3' flanking the deletion mutation, or the joining region between a sequence 5' flanking a potential STOP codon or splice site mutation as indicated above and the sequence of the potential STOP codon or splice site mutation), provided the mentioned 3'-located nucleotides are not derived exclusively from either the mutation region or flanking regions.

It will also be immediately clear to the skilled artisan that properly selected PCR primer pairs should also not comprise sequences complementary to each other.

For the purpose of the invention, the "complement of a nucleotide sequence represented in SEQ ID NO: X" is the nucleotide sequence which can be derived from the represented nucleotide sequence by replacing the nucleotides through their complementary nucleotide according to Chargaff's rules (A⇔T; G⇔C) and reading the sequence in the 5' to 3' direction, i.e. in opposite direction of the represented nucleotide sequence.

Examples of primers suitable to identify specific mutant GLUC1.1 alleles are described in the Examples.

As used herein, "the nucleotide sequence of SEQ ID No. Z from position X to position Y" indicates the nucleotide sequence including both nucleotide endpoints.

Preferably, the amplified fragment has a length of between 50 and 1000 nucleotides, such as a length between 50 and 500 nucleotides, or a length between 100 and 350 nucleotides. The specific primers may have a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region, a sequence within the mutation region, or a sequence spanning the joining region between the 3' or 5' flanking and mutation regions of the specific mutant GLUC1.1 allele, provided the mismatches still allow specific identification of the specific mutant GLUC1.1 allele with these primers under optimized PCR conditions. The range of allowable mismatches however, can easily be determined experimentally and are known to a person skilled in the art.

Detection and/or identification of a "mutant GLUC1.1 specific fragment" can occur in various ways, e.g., via size estimation after gel or capillary electrophoresis or via fluorescence-based detection methods. The mutant GLUC1.1 specific fragments may also be directly sequenced. Other sequence specific methods for detection of amplified DNA fragments are also known in the art.

Standard PCR protocols are described in the art, such as in 'PCR Applications Manual" (Roche Molecular Biochemicals, 2nd Edition, 1999) and other references. The optimal conditions for the PCR, including the sequence of the specific primers, is specified in a "PCR identification protocol" for each specific mutant GLUC1.1 allele. It is however understood that a number of parameters in the PCR identification protocol may need to be adjusted to specific laboratory conditions, and may be modified slightly to obtain similar results. For instance, use of a different method for preparation of DNA may require adjustment of, for instance, the amount of primers, polymerase, $MgCl_2$ concentration or annealing conditions used. Similarly, the selection of other primers may dictate other optimal conditions for the PCR identification protocol. These adjustments will however be apparent to a person skilled in the art, and are furthermore detailed in current PCR application manuals such as the one cited above.

Examples of PCR identification protocols to identify specific mutant GLUC1.1 alleles are described in the Examples.

Alternatively, specific primers can be used to amplify a mutant GLUC1.1 specific fragment that can be used as a "specific probe" for identifying a specific mutant GLUC1.1 allele in biological samples. Contacting nucleic acid of a biological sample, with the probe, under conditions which allow hybridization of the probe with its corresponding fragment in the nucleic acid, results in the formation of a nucleic acid/probe hybrid. The formation of this hybrid can be detected (e.g. labeling of the nucleic acid or probe), whereby the formation of this hybrid indicates the presence of the specific mutant GLUC1.1 allele. Such identification methods based on hybridization with a specific probe (either on a solid phase carrier or in solution) have been described in the art. The specific probe is preferably a sequence which, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region and/or within the mutation region of the specific mutant GLUC1.1 allele (hereinafter referred to as "GLUC1.1 mutation specific region"). Preferably, the specific probe comprises a sequence of between 20 and 1000 bp, 50 and 600 bp, between 100 to 500 bp, between 150 to 350 bp, which is at least 80%, preferably between 80 and 85%, more preferably between 85 and 90%, especially preferably between 90 and 95%, most preferably between 95% and 100% identical (or complementary) to the nucleotide sequence of a specific region. Preferably, the specific probe will comprise a sequence of about 15 to about 100 contiguous nucleotides identical (or complementary) to a specific region of the specific mutant GLUC1.1 allele.

Specific probes suitable for the invention may be the following:

- oligonucleotides ranging in length from 20 nt to about 1000 nt, comprising a nucleotide sequence of at least 20 consecutive nucleotides selected from the 5' flanking sequence of a specific mutant GLUC1.1 allele (i.e., for example, the sequence 5' flanking the one or more nucleotides deleted, inserted or substituted in the mutant GLUC1.1 alleles of the invention, such as the sequence 5' flanking the deletion, non-sense or splice site mutations described above or the sequence 5' flanking the potential STOP codon or splice site mutations indicated above), or a sequence having at least 80% sequence identity therewith (probes recognizing 5' flanking sequences); or
- oligonucleotides ranging in length from 20 nt to about 1000 nt, comprising a nucleotide sequence of at least 20 consecutive nucleotides selected from the 3' flanking sequence of a specific mutant GLUC1.1 allele (i.e., for example, the sequence 3' flanking the one or more nucleotides deleted, inserted or substituted in the mutant GLUC1.1 alleles of the invention, such as the sequence 3' flanking the deletion, non-sense or splice site mutations described above or the sequence 3' flanking the potential STOP codon or splice site mutations indicated above), or a sequence having at least 80% sequence identity therewith (probes recognizing 3' flanking sequences); or
- oligonucleotides ranging in length from 20 nt to about 1000 nt, comprising a nucleotide sequence of at least 20 consecutive nucleotides selected from the mutation sequence of a specific mutant GLUC1.1 allele (i.e., for example, the sequence of nucleotides inserted or substituted in the GLUC1.1 genes of the invention, or the complement thereof), or a sequence having at least 80% sequence identity therewith (probes recognizing mutation sequences).

The probes may entirely consist of nucleotide sequence selected from the mentioned nucleotide sequences of flanking and mutation sequences. However, the nucleotide sequence of the probes at their 5' or 3' ends is less critical. Thus, the 5' or 3' sequences of the probes may consist of a nucleotide sequence selected from the flanking or mutation sequences, as appropriate, but may consist of a nucleotide sequence unrelated to the flanking or mutation sequences. Such unrelated sequences should preferably be not longer than 50, more preferably not longer than 25 or even not longer than 20 or 15 nucleotides.

Moreover, suitable probes may comprise or consist of a nucleotide sequence spanning the joining region between flanking and mutation sequences (i.e., for example, the joining region between a sequence 5' flanking one or more nucleotides deleted, inserted or substituted in the mutant GLUC1.1 alleles of the invention and the sequence of the one or more nucleotides inserted or substituted or the sequence 3' flanking the one or more nucleotides deleted, such as the joining region between a sequence 5' flanking deletion, non-sense or splice site mutations in the GLUC1.1 genes of the invention described above and the sequence of the non-sense or splice site mutations or the sequence 3' flanking the deletion mutation, or the joining region between a sequence 5' flanking a potential STOP codon or splice site mutation indicated above and the sequence of the potential STOP codon or splice site mutation), provided the mentioned nucleotide sequence is not derived exclusively from either the mutation region or flanking regions.

Examples of specific probes suitable to identify specific mutant GLUC1.1 alleles are described in the Examples.

Detection and/or identification of a "mutant GLUC1.1 specific region" hybridizing to a specific probe can occur in various ways, e.g., via size estimation after gel electrophoresis or via fluorescence-based detection methods. Other sequence specific methods for detection of a "mutant GLUC1.1 specific region" hybridizing to a specific probe are also known in the art.

Alternatively, plants or plant parts comprising one or more mutant gluc1.1 alleles can be generated and identified using other methods, such as the "Delete-a-gene™" method which uses PCR to screen for deletion mutants generated by fast neutron mutagenesis (reviewed by Li and Zhang, 2002, Funct Integr Genomics 2:254-258), by the TILLING (Targeting Induced Local Lesions IN Genomes) method which identifies

EMS-induced point mutations using denaturing high-performance liquid chromatography (DHPLC) to detect base pair changes by heteroduplex analysis (McCallum et al., 2000, Nat Biotech 18:455, and McCallum et al. 2000, Plant Physiol. 123, 439-442), etc. As mentioned, TILLING uses high-throughput screening for mutations (e.g. using Cel 1 cleavage of mutant-wildtype DNA heteroduplexes and detection using a sequencing gel system). Thus, the use of TILLING to identify plants, seeds and tissues comprising one or more mutant gluc1.1 alleles in one or more tissues and methods for generating and identifying such plants is encompassed herein. Thus in one embodiment, the method according to the invention comprises the steps of mutagenizing plant seeds (e.g. EMS mutagenesis), pooling of plant individuals or DNA, PCR amplification of a region of interest, heteroduplex formation and high-throughput detection, identification of the mutant plant, sequencing of the mutant PCR product. It is understood that other mutagenesis and selection methods may equally be used to generate such mutant plants.

Instead of inducing mutations in GLUC1.1 alleles, natural (spontaneous) mutant alleles may be identified by methods known in the art. For example, ECOTILLING may be used (Henikoff et al. 2004, Plant Physiology 135(2):630-6) to screen a plurality of plants or plant parts for the presence of natural mutant gluc1.1 alleles. As for the mutagenesis techniques above, preferably *Gossypium* species are screened which comprise an A and/or a D genome, so that the identified gluc1.1 allele can subsequently be introduced into other *Gossypium* species, such as *Gossypium hirsutum*, by crossing (inter- or intraspecific crosses) and selection. In ECOTILLING natural polymorphisms in breeding lines or related species are screened for by the TILLING methodology described above, in which individual or pools of plants are used for PCR amplification of the gluc1.1 target, heteroduplex formation and high-throughput analysis. This can be followed up by selecting individual plants having a required mutation that can be used subsequently in a breeding program to incorporate the desired mutant allele.

The identified mutant alleles can then be sequenced and the sequence can be compared to the wild type allele to identify the mutation(s). Optionally functionality can be tested by expression in a homologous or heterologous host and testing the mutant GLUC1.1 protein for functionality in an enzyme assay. Using this approach a plurality of mutant gluc1.1 alleles (and *Gossypium* plants comprising one or more of these) can be identified. The desired mutant alleles can then be combined with the desired wild type alleles by crossing and selection methods as described further below. Finally a single plant comprising the desired number of mutant gluc1.1 and the desired number of wild type GLUC1.1 alleles is generated.

Oligonucleotides suitable as PCR primers or specific probes for detection of a specific mutant GLUC1.1 allele can also be used to develop methods to determine the zygosity status of the specific mutant GLUC1.1 allele.

To determine the zygosity status of a specific mutant GLUC1.1 allele, a PCR-based assay can be developed to determine the presence of a mutant and/or corresponding wild type GLUC1.1 specific allele:

To determine the zygosity status of a specific mutant GLUC1.1 allele, two primers specifically recognizing the wild-type GLUC1.1 allele can be designed in such a way that they are directed towards each other and have the mutation region located in between the primers. These primers may be primers specifically recognizing the 5' and 3' flanking sequences, respectively. This set of primers allows simultaneous diagnostic PCR amplification of the mutant, as well as of the corresponding wild type GLUC1.1 allele.

Alternatively, to determine the zygosity status of a specific mutant GLUC1.1 allele, two primers specifically recognizing the wild-type GLUC1.1 allele can be designed in such a way that they are directed towards each other and that one of them specifically recognizes the mutation region. These primers may be primers specifically recognizing the sequence of the 5' or 3' flanking region and the mutation region of the wild type GLUC1.1 allele, respectively. This set of primers, together with a third primer which specifically recognizes the sequence of the mutation region in the mutant GLUC1.1 allele, allow simultaneous diagnostic PCR amplification of the mutant GLUC1.1 gene, as well as of the wild type GLUC1.1 gene.

Alternatively, to determine the zygosity status of a specific mutant GLUC1.1 allele, two primers specifically recognizing the wild-type GLUC1.1 allele can be designed in such a way that they are directed towards each other and that one of them specifically recognizes the joining region between the 5' or 3' flanking region and the mutation region. These primers may be primers specifically recognizing the 5' or 3' flanking sequence and the joining region between the mutation region and the 3' or 5' flanking region of the wild type GLUC1.1 allele, respectively. This set of primers, together with a third primer which specifically recognizes the joining region between the mutation region and the 3' or 5' flanking region of the mutant GLUC1.1 allele, respectively, allow simultaneous diagnostic PCR amplification of the mutant GLUC1.1 gene, as well as of the wild type GLUC1.1 gene.

Alternatively, the zygosity status of a specific mutant GLUC1.1 allele can be determined by using alternative primer sets which specifically recognize mutant and wild type GLUC1.1 alleles.

If the plant is homozygous for the mutant GLUC1.1 gene or the corresponding wild type GLUC1.1 gene, the diagnostic PCR assays described above will give rise to a single PCR product typical, preferably typical in length, for either the mutant or wild type GLUC1.1 allele. If the plant is hemizygous for the mutant GLUC1.1 allele, two specific PCR products will appear, reflecting both the amplification of the mutant and the wild type GLUC1.1 allele.

Identification of the wild type and mutant GLUC1.1 specific PCR products can occur e.g. by size estimation after gel or capillary electrophoresis (e.g. for mutant GLUC1.1 alleles comprising a number of inserted or deleted nucleotides which results in a size difference between the fragments amplified from the wild type and the mutant GLUC1.1 allele, such that said fragments can be visibly separated on a gel); by evaluating the presence or absence of the two different fragments after gel or capillary electrophoresis, whereby the diagnostic PCR amplification of the mutant GLUC1.1 allele can, optionally, be performed separately from the diagnostic PCR amplification of the wild type GLUC1.1 allele; by direct sequencing of the amplified fragments; or by fluorescence-based detection methods.

Examples of primers suitable to determine the zygosity of specific mutant GLUC1.1 alleles are described in the Examples.

Alternatively, to determine the zygosity status of a specific mutant GLUC1.1 allele, a hybridization-based assay can be developed to determine the presence of a mutant and/or corresponding wild type GLUC1.1 specific allele:

To determine the zygosity status of a specific mutant GLUC1.1 allele, two specific probes recognizing the wild-type GLUC1.1 allele can be designed in such a way that each probe specifically recognizes a sequence within the

GLUC1.1 wild type allele and that the mutation region is located in between the sequences recognized by the probes. These probes may be probes specifically recognizing the 5' and 3' flanking sequences, respectively. The use of one or, preferably, both of these probes allows simultaneous diagnostic hybridization of the mutant, as well as of the corresponding wild type GLUC1.1 allele.

Alternatively, to determine the zygosity status of a specific mutant GLUC1.1 allele, two specific probes recognizing the wild-type GLUC1.1 allele can be designed in such a way that one of them specifically recognizes a sequence within the GLUC1.1 wild type allele upstream or downstream of the mutation region, preferably upstream of the mutation region, and that one of them specifically recognizes the mutation region. These probes may be probes specifically recognizing the sequence of the 5' or 3' flanking region, preferably the 5' flanking region, and the mutation region of the wild type GLUC1.1 allele, respectively. The use of one or, preferably, both of these probes, optionally, together with a third probe which specifically recognizes the sequence of the mutation region in the mutant GLUC1.1 allele, allow diagnostic hybridization of the mutant and of the wild type GLUC1.1 gene.

Alternatively, to determine the zygosity status of a specific mutant GLUC1.1 allele, a specific probe recognizing the wild-type GLUC1.1 allele can be designed in such a way that the probe specifically recognizes the joining region between the 5' or 3' flanking region, preferably the 5' flanking region, and the mutation region of the wild type GLUC1.1 allele. This probe, optionally, together with a second probe which specifically recognizes the joining region between the 5' or 3' flanking region, preferably the 5' flanking region, and the mutation region of the mutant GLUC1.1 allele, allows diagnostic hybridization of the mutant and of the wild type GLUC1.1 gene.

Alternatively, the zygosity status of a specific mutant GLUC1.1 allele can be determined by using alternative sets of probes which specifically recognize mutant and wild type GLUC1.1 alleles.

If the plant is homozygous for the mutant GLUC1.1 gene or the corresponding wild type GLUC1.1 gene, the diagnostic hybridization assays described above will give rise to a single specific hybridization product, such as one or more hybridizing DNA (restriction) fragments, typical, preferably typical in length, for either the mutant or wild type GLUC1.1 allele. If the plant is hemizygous for the mutant GLUC1.1 allele, two specific hybridization products will appear, reflecting both the hybridization of the mutant and the wild type GLUC1.1 allele.

Identification of the wild type and mutant GLUC1.1 specific hybridization products can occur e.g. by size estimation after gel or capillary electrophoresis (e.g. for mutant GLUC1.1 alleles comprising a number of inserted or deleted nucleotides which results in a size difference between the hybridizing DNA (restriction) fragments from the wild type and the mutant GLUC1.1 allele, such that said fragments can be visibly separated on a gel); by evaluating the presence or absence of the two different specific hybridization products after gel or capillary electrophoresis, whereby the diagnostic hybridization of the mutant GLUC1.1 allele can, optionally, be performed separately from the diagnostic hybridization of the wild type GLUC1.1 allele; by direct sequencing of the hybridizing DNA (restriction) fragments; or by fluorescence-based detection methods.

Examples of probes suitable to determine the zygosity of specific mutant GLUC1.1 alleles are described in the Examples.

Furthermore, detection methods specific for a specific mutant GLUC1.1 allele which differ from PCR- or hybridization-based amplification methods can also be developed using the specific mutant GLUC1.1 allele specific sequence information provided herein. Such alternative detection methods include linear signal amplification detection methods based on invasive cleavage of particular nucleic acid structures, also known as Invader™ technology, (as described e.g. in U.S. Pat. No. 5,985,557 "Invasive Cleavage of Nucleic Acids", U.S. Pat. No. 6,001,567 "Detection of Nucleic Acid sequences by Invader Directed Cleavage, incorporated herein by reference), RT-PCR-based detection methods, such as Taqman, or other detection methods, such as SNPlex.

In another aspect of the invention, kits are provided. A "kit" as used herein refers to a set of reagents for the purpose of performing the methods of the invention, more particularly, the identification of a specific mutant GLUC1.1 allele in biological samples or the determination of the zygosity status of plant material comprising a specific mutant GLUC1.1 allele. More particularly, a preferred embodiment of the kit of the invention comprises at least two specific primers, as described above, for identification of a specific mutant GLUC1.1 allele, or at least two or three specific primers for the determination of the zygosity status. Optionally, the kit can further comprise any other reagent described herein in the PCR identification protocol. Alternatively, according to another embodiment of this invention, the kit can comprise at least one specific probe, which specifically hybridizes with nucleic acid of biological samples to identify the presence of a specific mutant GLUC1.1 allele therein, as described above, for identification of a specific mutant GLUC1.1 allele, or at least two or three specific probes for the determination of the zygosity status. Optionally, the kit can further comprise any other reagent (such as but not limited to hybridizing buffer, label) for identification of a specific mutant GLUC1.1 allele in biological samples, using the specific probe.

The kit of the invention can be used, and its components can be specifically adjusted, for purposes of quality control (e.g., purity of seed lots), detection of the presence or absence of a specific mutant GLUC1.1 allele in plant material or material comprising or derived from plant material, such as but not limited to cotton seeds, raw cotton, cotton bales, yarn, fabric, apparel, etc.

The term "primer" as used herein encompasses any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process, such as PCR. Typically, primers are oligonucleotides from 10 to 30 nucleotides, but longer sequences can be employed. Primers may be provided in double-stranded form, though the single-stranded form is preferred. Probes can be used as primers, but are designed to bind to the target DNA or RNA and need not be used in an amplification process.

The term "recognizing" as used herein when referring to specific primers, refers to the fact that the specific primers specifically hybridize to a nucleic acid sequence in a specific mutant GLUC1.1 allele under the conditions set forth in the method (such as the conditions of the PCR identification protocol), whereby the specificity is determined by the presence of positive and negative controls.

The term "hybridizing" as used herein when referring to specific probes, refers to the fact that the probe binds to a specific region in the nucleic acid sequence of a specific mutant GLUC1.1 allele under standard stringency conditions. Standard stringency conditions as used herein refers to the conditions for hybridization described herein or to the conventional hybridizing conditions as described by Sambrook et al., 1989 (Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbour Laboratory Press, NY) which for instance can comprise the following steps: 1) immobilizing plant genomic DNA fragments or BAC library DNA on a filter, 2) prehybridizing the filter for 1 to 2 hours at 65° C. in 6×SSC, 5×Denhardt's reagent, 0.5% SDS and 20 μg/ml denaturated carrier DNA, 3) adding the hybridization probe which has been labeled, 4) incubating for 16 to 24 hours, 5) washing the filter once for 30 min. at 68° C. in 6×SSC, 0.1% SDS, 6) washing the filter three times (two times for 30 min. in 30 ml and once for 10 min in 500 ml) at 68° C. in 2×SSC, 0.1% SDS, and 7) exposing the filter for 4 to 48 hours to X-ray film at −70° C.

As used in herein, a "biological sample" is a sample of a plant, plant material or product comprising plant material. The term "plant" is intended to encompass *Gossypium* plant tissues, at any stage of maturity, as well as any cells, tissues, or organs taken from or derived from any such plant, including without limitation, any fibers, seeds, leaves, stems, flowers, roots, single cells, gametes, cell cultures, tissue cultures or protoplasts. "Plant material", as used herein refers to material which is obtained or derived from a plant. Products comprising plant material relate to food, feed or other products, such as raw cotton, cotton bales, yarn, fabric, apparel, etc., which are produced using plant material or can be contaminated by plant material. It is understood that, in the context of the present invention, such biological samples are tested for the presence of nucleic acids specific for a specific mutant GLUC1.1 allele, implying the presence of nucleic acids in the samples. Thus the methods referred to herein for identifying a specific mutant GLUC1.1 allele in biological samples, relate to the identification in biological samples of nucleic acids which comprise the specific mutant GLUC1.1 allele.

The present invention also relates to the transfer of one or more specific mutant GLUC1.1 allele(s) in one *Gossypium* plant to another *Gossypium* plant, to the combination of specific GLUC1.1 alleles in one plant, to the plants comprising one or more specific mutant GLUC1.1 allele(s), the progeny obtained from these plants and to the plant cells, or plant material derived from these plants.

Thus, in one embodiment of the invention a method for transferring a non-functionally expressed GLUC1.1 allele from one *Gossypium* plant to another *Gossypium* plant is provided comprising the steps of:

(a) crossing a *Gossypium* plant comprising a non-functionally expressed GLUC1.1 allele, as described above, with a second *Gossypium* plant,
(b) collecting F1 hybrid seeds from the cross,
(c) optionally, backcrossing the F1 plants, derived from the F1 seeds, for one or more generations (x), collecting BCx seeds from the crosses, and identifying in every generation BCx plants, derived from the BCx seeds, comprising the non-functionally expressed GLUC1.1 allele as described above,
(d) selfing the F1 or BCx plants, derived from the F1 or BCx seeds,
(e) collecting F1 S1 or BCx S1 seeds from the selfing,
(f) identifying F1 S1 or BCx S1 plants, derived from the F1 S1 or BCx S1 seeds, comprising the non-functionally expressed GLUC1.1 allele as described above.

In another embodiment of the invention a method for combining at least two non-functionally expressed GLUC1.1 alleles in one *Gossypium* plant is provided comprising the steps of:

(a) transferring a non-functionally expressed GLUC1.1 allele(s) from one *Gossypium* plant to another *Gossypium* plant as described above,
(b) repeating step (a) until the desired number and/or types of non-functionally expressed GLUC1.1 alleles are combined in the second plant.

In yet another embodiment of the invention, a method is provided for altering the callose content of a fiber in a fiber producing plant, such as *Gossypium* plants, comprising the steps of:

(a) abolishing the functional expression of at least one allele of at least one fiber specific GLUC gene that is functionally expressed during the fiber strength building phase of fiber development,
(b) identifying a plant, which produces fibers, the callose content of which is increased as compared to the callose content of the fibers of a corresponding plant in which the functional expression of the GLUC gene is not abolished.

In still another embodiment of the invention, a method is provided for altering the properties of a fiber, particularly increasing the strength of a fiber, in a fiber producing plant, such as a *Gossypium* plant, comprising the steps of:

(c) abolishing the functional expression of at least one allele of at least one fiber specific GLUC gene that is functionally expressed during the fiber strength building phase of fiber development,
(d) identifying a plant, which produces fibers, the strength of which is increased as compared to the strength of fibers of a corresponding plant in which the functional expression of the GLUC gene is not abolished.

In another aspect of the invention, plant fibers with increased fiber strength are are provided derived from fiber-producing plants according to the invention, especially of *Gossypium hirsutum* plants as provided herein, but also from other *Gossypium* species. For example, *Gossypium* species wherein the expression of at least one fiber specific GLUC gene that is functionally expressed during the fiber strength building phase of fiber development, such as a GLUC1.1A and/or GLUC1.1D gene, can be abolished, for example *Gossypium tomentosum, Gossypium mustilinum, Gossypium herbaceum*, or *Gossypium raimondii.*

Also included in the invention is the use of the fibers of this invention, for example, in the production of raw cotton, cotton bales, yarn, fabric, apparel, etc.

Other applications, such as mixing fibers with a specific callose content and/or a specific modified strength according to the invention with other fibers with a lower callose content and/or a lower fiber to increase the average callose content and/or fiber strength in, for example, cotton bales, yarn, fabric, apparel, etc; thus making it more suitable for certain applications, such as but not limited to, the production of biodiesel, stronger textile, etc., are also included in the invention.

It will be clear that whenever nucleotide sequences of RNA molecules are defined by reference to nucleotide sequence of corresponding DNA molecules, the thymine (T) in the nucleotide sequence should be replaced by uracil (U). Whether reference is made to RNA or DNA molecules will be clear from the context of the application.

It is understood that when referring to a word in the singular (e.g. plant or root), the plural is also included herein (e.g. a plurality of plants, a plurality of roots). Thus, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

As used herein "comprising" is to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. Thus, e.g., a nucleic acid or protein comprising a sequence of nucleotides or amino acids, may comprise more nucleotides or amino acids than the actually cited ones, i.e., be embedded in a larger nucleic acid or protein. A chimeric gene comprising a DNA region, which is functionally or structurally defined, may comprise additional DNA regions etc. A plant comprising a certain trait may thus comprise additional traits etc.

The following non-limiting Examples describe the identification of a fiber strength locus on chromosome A05 in cotton and the characterization of a GLUC1.1 gene located in the 1-LOD support interval of the Strength QTL. Unless stated otherwise in the Examples, all recombinant DNA techniques are carried out according to standard protocols as described in Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, NY and in Volumes 1 and 2 of Ausubel et al. (1994) Current Protocols in Molecular Biology, Current Protocols, USA. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfax (1993) by R. D. D. Croy, jointly published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications, UK.

Throughout the description and Examples, reference is made to the following sequences represented in the sequence listing:

SEQ ID NO: 1: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium hirsutum* cv. Fiber Max966, A-subgenome specific

SEQ ID NO: 2: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 1

SEQ ID NO: 3: amplified cDNA fragment of endo-1,3-beta-glucanase gene from *Gossypium hirsutum* cv. Fiber Max966, A-subgenome specific

SEQ ID NO: 4: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 3

SEQ ID NO: 5: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium barbadense* cv. PimaS7, A-subgenome specific

SEQ ID NO: 6: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 5

SEQ ID NO: 7: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium hirsutum* cv. Fiber Max966, D-subgenome specific

SEQ ID NO: 8: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 7

SEQ ID NO: 9: amplified cDNA fragment of endo-1,3-beta-glucanase gene from *Gossypium hirsutum* cv. Fiber Max966, D-subgenome specific

SEQ ID NO: 10: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 9

SEQ ID NO: 11: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium barbadense* cv. PimaS7, D-subgenome specific

SEQ ID NO: 12: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 11

SEQ ID NO: 13: amplified cDNA fragment of endo-1,3-beta-glucanase gene from *Gossypium barbadense* cv. PimaS7, D-subgenome specific

SEQ ID NO: 14: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 13

SEQ ID NO: 15: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium tomentosum*, A-subgenome specific

SEQ ID NO: 16: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 15

SEQ ID NO: 17: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium darwinii*, A-subgenome specific

SEQ ID NO: 18: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 17

SEQ ID NO: 19: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium* mustelinum, A-subgenome specific

SEQ ID NO: 20: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 19

SEQ ID NO: 21: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium arboreum*, A-subgenome specific

SEQ ID NO: 22: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 21

SEQ ID NO: 23: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium herbaceum*, A-subgenome specific

SEQ ID NO: 24: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 23

SEQ ID NO: 25: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium tomentosum*, D-subgenome specific

SEQ ID NO: 26: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 25

SEQ ID NO: 27: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium darwinii*, D-subgenome specific

SEQ ID NO: 28: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 27

SEQ ID NO: 29: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium mustelinum*, D-subgenome specific

SEQ ID NO: 30: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 29

SEQ ID NO: 31: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium raimondii*, D-subgenome specific

SEQ ID NO: 32: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 31

SEQ ID NO: 33: forward primer SE077 for amplification of endo-1,3-beta-glucanase genomic fragment

SEQ ID NO: 34: reverse primer SE078 for amplification of endo-1,3-beta-glucanase genomic fragment

SEQ ID NO: 35: forward primer SE002 for amplification of endo-1,3-beta-glucanase genomic fragment

SEQ ID NO: 36: reverse primer SE003 for amplification of endo-1,3-beta-glucanase genomic fragment

SEQ ID NO: 37: forward primer p1.3GlucaAf for amplification of endo-1,3-beta-glucanase genomic fragment, in particular for discriminating different variants of polymorphic site GLUC1.1A-SNP2

SEQ ID NO: 38: reverse primer p1.3GlucaAr for amplification of endo-1,3-beta-glucanase genomic fragment, in particular for discriminating different variants of polymorphic site GLUC1.1A-SNP2

SEQ ID NO: 39: probe TM249-GCM1 for detecting the *G. barbadense* variant of polymorphic site GLUC1.1A-SNP3

SEQ ID NO: 40: probe TM249-GCV1 for detecting the *G. hirsutum* variant of polymorphic site GLUC1.1A-SNP3

SEQ ID NO: 41: forward primer TM249-GCF for amplification of endo-1,3-beta-glucanase genomic fragment, in particular for discriminating different variants of polymorphic site GLUC1.1A-SNP3

SEQ ID NO: 42: reverse primer TM249-GCR for amplification of endo-1,3-beta-glucanase genomic fragment, in particular for discriminating different variants of polymorphic site GLUC1.1A-SNP3

SEQ ID NO: 43: AFLP primer P5 for amplification of genomic DNA fragment corresponding to marker P5M50-M126.7, in particular for discriminating different variants of marker P5M50-M126.7

SEQ ID NO: 44: AFLP primer M50 for amplification of genomic DNA fragment corresponding to marker P5M50-M126.7, in particular for discriminating different variants of marker P5M50-M126.7

SEQ ID NO: 45: forward SSR primer for amplification of genomic DNA fragment corresponding to marker NAU861, in particular for discriminating different variants of marker NAU861

SEQ ID NO: 46: reverse SSR primer for amplification of genomic DNA fragment corresponding to marker NAU861, in particular for discriminating different variants of marker NAU861

SEQ ID NO: 47: forward SSR primer for amplification of genomic DNA fragment corresponding to marker CIR401, in particular for discriminating different variants of marker CIR401

SEQ ID NO: 48: reverse SSR primer for amplification of genomic DNA fragment corresponding to marker CIR401, in particular for discriminating different variants of marker CIR401

SEQ ID NO: 49: forward SSR primer for amplification of genomic DNA fragment corresponding to marker BNL3992, in particular for discriminating different variants of marker BNL3992

SEQ ID NO: 50: reverse SSR primer for amplification of genomic DNA fragment corresponding to marker BNL3992, in particular for discriminating different variants of marker BNL3992

SEQ ID NO: 51: forward SSR primer for amplification of genomic DNA fragment corresponding to marker CIR280, in particular for discriminating different variants of marker CIR280

SEQ ID NO: 52: reverse SSR primer for amplification of genomic DNA fragment corresponding to marker CIR280, in particular for discriminating different variants of marker CIR280

SEQ ID NO: 53: DNA sequence of a 165250 bps DNA fragment spanning the GLUC1.1A gene in *G. hirsutum*

SEQ ID NO: 54: amplified cDNA fragment of endo-1,3-beta-glucanase gene from *Gossypium barbadense* cv. PimaS7, A-subgenome specific

SEQ ID NO: 55: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 54

SEQ ID NO: 56: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium darwinii*, A-subgenome specific

SEQ ID NO: 57: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 56

SEQ ID NO: 58: amplified genomic DNA fragment of endo-1,3-beta-glucanase gene from *Gossypium darwinii*, D-subgenome specific

SEQ ID NO: 59: endo-1,3-beta-glucanase protein encoded by SEQ ID NO: 58

SEQ ID NO: 60: probe for detecting the *G. barbadense* variant of polymorphic site GLUC1.1A-SNP5

SEQ ID NO: 61: probe for detecting the *G. hirsutum* variant of polymorphic site GLUC1.1A-SNP5



SEQ ID NO: 62: forward primer for amplification of endo-1,3-beta-glucanase genomic fragment, in particular for discriminating different variants of polymorphic site GLUC1.1A-SNP5

SEQ ID NO: 63: reverse primer for amplification of endo-1,3-beta-glucanase genomic fragment, in particular for discriminating different variants of polymorphic site GLUC1.1A-SNP5

SEQ ID NO: 64: forward primer G1.1-SGA-F for amplification of endo-1,3-beta-glucanase genomic fragment

SEQ ID NO: 65: forward primer G1.1-f1-F1 for amplification of endo-1,3-beta-glucanase genomic fragment

## EXAMPLES

### Example 1

### Identification and Characterization of a Quantitative Trait Locus (QTL) on Cotton Chromosome A05 Linked to Fiber Strength

1.1. QTL Discovery

Discovery of quantitative trait loci associated with cotton fiber properties was performed according to standard procedures. Briefly, parental cotton plant lines with fiber phenotypes of interest were selected, segregating populations were generated and the impact of the presence of specific chromosomal regions on measurable cotton fiber phenotypes was determined. The parental lines were *Gossypium hirsutum* cv. FM966 (used as female parent in the initial cross; abbreviated hereinafter as "FM"; particularly known for its high fiber yield, but lower fiber quality compared to *Gossypium barbadense* varieties) and *Gossypium barbadense* cv. PimaS7 (used as male parent in the initial cross; abbreviated hereinafter as "Pima"; particularly known for its excellent fiber quality, but lower fiber yield compared to *Gossypium hirsutum* varieties). Backcross populations with both parental lines were generated and evaluated in the greenhouse as well as in the field.

1.2. Evaluation of Plants Derived from a First Backcross to the *Gossypium barbadense* Pima S7 Parental Line ("Pima BC1F1 Population")

A QTL for fiber strength on chromosome A05 was originally detected in a BC1F1 mapping population [(FM×Pima)×Pima; recurrent parent used as male parent] of 119 individuals. The population was grown under standard growing conditions in a greenhouse. A genome-wide genetic map of about 800 markers was constructed based on amplified fragment length polymorphism PCR (AFLP-PCR or AFLP) marker data and simple sequence repeat (SSR or microsatellite) marker data from the 119 individuals using JoinMap software (map version 8 and 13; Stam, 1993, Plant J 3: 739-744). Fiber strength was measured by High-Volume Instruments (HVI) (United States Department of Agriculture, Agricultural Marketing Service) on samples from 88 of the 119 individual plants. QTL mapping was performed using MapQTL software (Van Ooijen and Maliepaard, 1996, Plant Genome IV Abstracts, World Wide Web site: intl-pag.org). Final QTL data are based on the restricted multiple QTL mapping (rMQM; Jansen, 1993, Genetics 135:205-211; Jansen and Stam, 1994, Genetics 136:1447-1455) analysis.

A clear QTL associated with fiber strength (also referred to as "Strength locus" or "Stren locus") was detected on chromosome A05. The QTL had a sharp LOD (logarithm of the odds) score peak with a maximum value of LOD 4.92 at a position of 98.61 cM from the tip of chromosome A05, with a 1-LOD support interval of 14 cM (from 91.515 cM to

105.61 cM). The 1-LOD QTL support interval was flanked by one AFLP marker, P5M50-M126.7, at 85.515 cM, and one microsatellite marker, CIR401c, at 109.13 cM. Within the QTL support interval one microsatellite marker NAU861 (94.61 cM) and a GLUC1.1 gene (94.602 cM) were located at close distance (ca 4 cM) to the position of maximum LOD value (Table 6). Primer pairs used to distinguish between the *G. hirsutum* and *G. barbadense* alleles of the markers are indicated in Table 2 above.

TABLE 6

Estimated position (according to JoinMap version 8 and 13) on chromosome A05 of markers linked to the fiber strength locus in the FM and Pima BC1F1 population

| Marker locus on chromosome A05 | Position (in cM as estimated with JoinMap version 8 or 13) on chromosome A05 of: FM BC1F1 map 8 | FM BC1F1 map 13 | Pima BC1F1 map 8 | Pima BC1F1 map 13 | 1-LOD support interval of Strength locus |
|---|---|---|---|---|---|
| P5M50-M126.7 | 104.582 | 107.9 | 85.515 | 105.5 | |
| | | | 91.515 | | Lower limit |
| GLUC1.1A | 107.599 | 111.1 | 94.602 | 114.6 | |
| NAU861 | 106.884 | 110.5 | 94.610 | 114.6 | |
| | | | 98.610 | | LOD Peak |
| | | | 105.610 | | upper limit |
| CIR401c | — | — | 109.130 | 129.1 | |
| CIR401b | 112.813 | 115.4 | — | — | |
| BNL3992 | 117.199 | 119.5 | nd | 132.1 | |
| CIR280 | nd | 124.5 | — | — | |

As indicated above, the GLUC1.1A gene was mapped within the support interval of the Strength locus (LOD of 4.431) using SNP marker GLUC1.1A-SNP2 as indicated in Table 13 and primers p1.3GlucaAf (SEQ ID NO: 37) and p1.3GlucaAr (SEQ ID NO: 38) as described in Example 6 below. Plants homozygous for the GLUC1.1A allele of *Gossypium barbadense* Pima S7 (Pima GLUC1.1A allele or Gbgluc1.1A) had 9.7% higher fiber strength compared to plants heterozygous for Gbgluc1.1A (Ho/He ratio of 109.7%). The QTL explained 17.8% of the variation for fiber strength in the population.

1.3. Evaluation of Plants Derived from a First Backcross to the *Gossypium hirsutum* FM966 Parental Line ("FM BC1F1 Population")

QTL mapping was also performed in a complementary BC1F1 population [(FM×Pima)×FM; recurrent parent used as male parent] of 130 individuals. Fiber strength was measured on samples from 94 of the 130 individual plants. The QTL for fiber strength in the region flanked by markers P5M50-M126.7 and CIR401 was not detected in this FM BC1F1 population (max LOD=0.42, i.e. below the critical threshold value of LOD=3). However, technically, plants heterozygous for the GLUC1.1A allele of *Gossypium barbadense* Pima S7 of this population did show about 1 to about 2% higher fiber strength compared to plants homozygous for the GLUC1.1A allele of *Gossypium hirsutum* FM966 (FM GLUC1.1A allele or GhGLUC1.1A). Together with the data from the Pima BC1F1 population this suggested that the GLUC1.1A allele of *Gossypium barbadense* Pima S7 provides superior fiber strength.

1.4. Evaluation of Plants Derived from a Fourth Backcross to the *Gossypium hirsutum* FM966 Parental Line ("FM BC4F1 Population")

With the purpose of improving fiber quality in *Gossypium hirsutum*, in particular in *Gossypium hirsutum* cv. FM966, genome fragments of the *Gossypium barbadense* parental line were backcrossed into the FM BC1F1 population by single seed descent and without selection during 4 generations (FM BC4F1 population). The Pima region of chromosome A05 carrying the candidate Strength locus was expected to be present in a number of these introgression lines.

A total of 219 FM BC4F1 plants originating from 75 FM BC3F1 plants (average 3 sister plants per line) were grown under standard growing conditions in a greenhouse. All plants were genotyped for 450 SSR markers and the strength of fibers from all plants was measured by HVI (see above). In the region of the Strength locus, 14 and 23 FM BC4F1 plants were heterozygous for the NAU861 and the GLUC1.1A markers, respectively, versus 196 and 194 plants that were homozygous for the NAU861 marker and the GLUC1.1A allele of *Gossypium hirsutum* FM966.

Table 7 summarizes the impact on fiber strength of the presence of different Pima marker alleles in heterozygous state versus the equivalent FM marker alleles in homozygous state (He/Ho ratio) in FM BC1F1 and FM BC4F1 populations. Markers indicated as CIRx, NAUx, JESPRx and BNLx are publicly available markers (see Cotton Microsatellite Database on the World Wide Web at cottonmarker.org). Markers indicated as 'Primer combination X and Y-amplified fragment size' are AFLP markers (Vos et al., 1995, NAR 23:4407-4414).

A similar effect on fiber strength was observed in both the FM BC1F1 and FM BC4F1 populations for the presence of the Pima GLUC1.1A allele (i.e. plants heterozygous for the Pima GLUC1.1A allele showed about 1 to about 2% higher fiber strength compared to plants homozygous for the FM GLUC1.1A allele).

TABLE 7

Estimated position (according to JoinMap version 8 and 13) on chromosome A05 and impact on fiber strength of different allele combinations (He versus Ho FM) for markers linked to the fiber strength locus in FM BC1F1 and FM BC4F1 populations

| Position (cM) vers. 13 | vers. 8 | Marker locus on chromosome A05 | FM BC1F1 K* | FM BC1F1 He/Ho(%) | FM BC4F1 K* | FM BC4F1 He/Ho(%) |
|---|---|---|---|---|---|---|
| 107.9 | 104.582 | P5M50-M126.7 | 1.926 | 102.52 | | |
| 110.5 | 106.884 | NAU861 | 1.334 | 102.09 | 2.189 | 102.60 |
| 111.1 | 107.599 | GLUC1.1A | 0.802 | 101.30 | 2.037 | 101.87 |
| 115.4 | 112.813 | CIR401b | 1.85 | 103.90 | 5.786 | 103.20 |
| 119.5 | 117.199 | BNL3992 | 1.329 | 103.32 | 5.786 | 103.20 |
| 124.5 | nd | CIR280 | nd | nd | nd | nd |

1.5. Evaluation of Plants Derived from the F2 Generation of a Fourth Backcross to the *Gossypium hirsutum* FM966 Parental Line ("FM BC4F2 Population")

As a next step, QTL validation in FM BC4F2 families was performed under field conditions in summer in Mississippi. FM BC4F2 plants segregate in 3 genetic classes: plants homozygous for FM marker alleles, plants homozygous for Pima marker alleles and plants heterozygous for FM and Pima marker alleles. In most cases 75-80 plants were genotyped per line and fiber samples from about 50 single plants were analyzed. This allowed testing of the effect of the FM or Pima marker alleles (and predicted linked genes) in heterozygous and homozygous condition.

The field trial included 4 FM BC4F2 families (called lines 6, 10, 20 and 94) segregating for various portions of the region of chromosome A05 carrying the Strength locus from Pima S7. Segregation was tested using 6 markers: BNL0542, BNL3995, CIR139a, NAU861, GLUC1.1A, BNL3992.

All BC4F2 plants of line 6 were homozygous for the FM allele of the markers tested. Line 94 produced only 38 FM BC4F2 plants and only 10 of those produced sufficient fiber for single plant analysis. The two remaining lines, lines 10 and 20, produced larger numbers of plants and had good marker segregation. Line 10 contained a segment of chromosome A05 of Pima carrying the Strength locus centered around the GLUC1.1 gene. The second line, line 20, contained a segment of chromosome A05 of Pima shifted to the lower end of the Strength locus support region.

In line 10 the expectation that plants homozygous for the Pima GLUC1.1A allele produce stronger fibers was confirmed. The fiber strength of plants homozygous for the Pima GLUC1.1A allele was on average 2.5 grams per tex higher than the fiber strength of plants homozygous for the FM GLUC1.1A allele (35.5 g/tex versus 33.0 g/tex or 7.5% increase in fiber strength). A similar result was observed for the two markers NAU861 and BNL3992 which are closely linked to GLUC1.1A on either side. The differences in fiber strength between homozygous FM plants, homozygous Pima plants and heterozygous plants were not significant in Anova, but they were significant in paired t-test between homozygous FM plants and the other two classes.

In line 20 the Pima alleles of markers NAU861 and BNL3992 did not provide stronger fiber. This line segregates for a lower section of the region of Pima chromosome A05, in the tail of the QTL support interval. This line also does not contain the Pima allele of the GLUC1.1A gene.

The data in Table 8 consolidate the results for line 10 in terms of "Marker Trait Performance" for fiber strength (MTP, calculated as ratio of the difference in average trait performance for two marker classes (HoFM-HoPima) and the average standard deviation for trait performance in both marker classes). It is shown that plants homozygous for the Pima allele of markers NAU861, GLUC1.1A and BNL3992 had stronger fibers than plants homozygous for the FM allele of these markers (negative MTP). However, the difference in performance was smaller than the average standard deviation (MTP value between 0 and −1).

Thus, the field trial data provide evidence in support of the idea that there is a QTL associated with fiber strength on chromosome A05, close to or coinciding with the GLUC1.1A gene, with the superior allele coming from *Gossypium barbadense* PimaS7.

Due to the low number of plants in the FM BC4F2 population it was not possible to fine map the QTL position. In this respect it is noted that the Pima allele of a marker (BNL3992) that was included in the introgressed Pima fragment in line 10, but resided at a position outside the original support interval on the Pima BC1F1 map also segregated with the enhanced fiber strength derived from PimaS7. This can be explained by the fact that in the original BC1 population sufficient recombinations had occurred to place this marker outside the QTL support interval, while in the (smaller) BC4F2 populations it remained linked to the QTL causal gene more frequently.

TABLE 8

Estimated position on chromosome A05 and impact on fiber strength (indicated as MTP) of different allele combinations (HH FM versus HH Pima) for markers linked to the Strength locus in FM BC4F2 plant lines

| Position (cM - vers. 8) | Marker locus on chromosome A05 | Graphical phenotype for marker of BC4F1 plants giving rise to FM BC4F2 plant line n° 6 | 10 | 20 | 94 | MTP for fiber strength in line n° 10 |
|---|---|---|---|---|---|---|
| 78.883 | CIR139a* | h | a | a | a | |
| 79.911 | BNL3029.A | h | a | a | a | |
| 82.969 | NAU1042.A | h | a | a | a | |
| 106.884 | NAU861* | h | h | a | h | −.70 |
| 107.599 | GLUC1.1A* | h | h | a | h | −.67 |
| 112.813 | CIR401c | h | h | a | h | −.55 |
| 117.199 | BNL3992* | h | h | a | h | |
| 136.15 | BNL0542* | a | a | h | h | |
| 146.257 | E43M49-M260.0 | a | a | h | h | |
| 149.542 | E31M48-M188.5 | a | a | h | a | |
| 159.609 | E43M53-M460.0 | a | a | h | a | |
| 161.272 | CIR294.A | a | a | h | a | |
| 163.129 | BNL3995* | a | a | h | a | |

Column 2 lists markers on chromosome A05 linked to the Strength locus. Markers indicated as CIRx, NAUx and BNLx are publicly available markers (see Cotton Microsatellite Database). Markers indicated as 'Primer combination X and Y-amplified fragment size' are AFLP markers (Vos et al., 1995, NAR 23:4407-4414). Column 1 indicates their map positions on the genetic map (in cM) of the FM BC1F1 mapping population constructed using JoinMap software map version 8. Graphical genotypes for the markers are indicated for BC4F1 plants that gave rise to BC4F2 families 6, 10, 20 and 94: a=homozygous FM966, h=heterozygous. Segregation of the 'h' regions in the graphical genotypes was investigated using marker data for markers indicated with *. Average phenotypic performance for fiber strength was compared for groups of plants homozygous for FM966 markers (genotype "HH FM") and for groups of plants homozygous for Pima markers (genotype "HH Pima"). Marker Trait Performance (MTP) is expressed as ((average phenotype HH FM—average phenotype HH Pima)/0.5×(SD HH FM+SD HH Pima)). Positive MTP means performance FM is higher than performance Pima. Negative MTP means performance Pima is higher than performance FM. MTP higher than 1 and MTP lower than −1 means delta performance exceeds average standard deviation (SD). Data for fiber strength properties are based on homozygous segregates among 60 plants.

Example 2

Identification and Characterization of a Glucanase Gene Linked to the Fiber Strength Locus on Cotton Chromosome A05

2.1 Characterization of the GLUCL1A Gene Localized in the Support Interval of the Strength Locus

As described in Example 1.2, a GLUC1.1 gene was mapped within the support interval of the predicted QTL for fiber strength on chromosome A05, suggesting that the GLUC1.1A candidate gene might be the causal gene for fiber strength. As further described in Example 1, the superior allele comes from the Pima parental line rather than from the FM parental line.

Based on the GhGLUC1.1A and D nucleotide sequences described in WO2008/083969 (SEQ ID NO: 1 and 7, respectively), 2 primers (forward primer SE077 (SEQ ID NO: 33) en reverse primer SE078 (SEQ ID NO: 34)) were designed to amplify genomic DNA fragments for *G. barbadense* (reaction mix and PCR conditions as described in Example 4). Two genomic DNA sequences were derived: one for GbGLUC1.1A (SEQ ID NO: 5) and one for GbGLUC1.1D (SEQ ID NO: 11).

The 2 primers (forward primer SE077 (SEQ ID NO: 33) en reverse primer SE078 (SEQ ID NO: 34)) were also used to amplify GLUC1.1A and GLUC1.1D cDNA from cDNA libraries from *G. hirsutum* and *G. barbadense* (reaction mix and PCR conditions as described in Example 4). cDNA sequences were derived for GhGLUC1.1A (SEQ ID NO: 3), for GhGLUC1.1D (SEQ ID NO: 9), and for GbGLUC1.1D (SEQ ID NO: 13). Forward primer G1.1-SGA-F (SEQ ID NO: 64) en reverse primer SE078 (SEQ ID NO: 34) were used to amplify GLUC1.1A cDNA from a cDNA libraries from *G. barbadense*. The cDNA sequence was derived for GbGLUC1.1A (SEQ ID NO: 54).

Alignment of genomic and cDNA sequences of A and D subgenome-specific GLUC1.1 genes from *Gossypium hirsutum* and *Gossypium barbadense* indicated that the GLUC1.1A gene from *Gossypium barbadense* displayed a c to t nucleotide substitution (at position 712 of SEQ ID NO: 5) that resulted in a putative premature STOP codon (cga to tga) as compared to the GLUC1.1A and D genes from *Gossypium hirsutum* and the GLUC1.1D gene from *Gossypium barbadense* (FIG. **1**), that is predicted to result in the production of a truncated GLUC1.1A protein in *Gossypium barbadense* (FIG. **2**). Compared to the *Gossypium hirsutum* ortholog, the *Gossypium barbadense* GLUC1.1A amino acid sequence lacks the GH17 signature (FIG. **2**).

2.2. Characterization of the GLUC1.1A Protein from Different *Gossypium* sp.

Protein modeling based on an X-ray structure of a barley 1,3-1,4-beta-glucanase belonging to the GH17 family of glycosidase hydrolases (laq0 in Protein Data Bank) (FIG. **3**, left), using FUGUE™ and ORCHESTRAR™ technologies from Sybyl7.3, showed that the GLUC1.1A protein of *G. barbadense* (FIG. **3***b*, right) is missing the active site and substrate binding cleft (located within the area indicated by the amino acids and their position numbers, displayed in the upper left part of the protein model of laq0 and described in Müller et al., 1998, Biol Chem 273: 3438-3446), which was found to be present in the GLUC1.1A and D proteins of *G. hirsutum* and in the GLUC1.1D protein of *G. barbadense* (FIG. **3***a*, right). The GLUC1.1A protein of *G. barbadense* is therefore predicted to be inactive.

2.3. Characterization of the Genomic Regions Spanning the GLUCL1 Alleles from Different *Gossypium* sp.

DNA sequencing of an about 165 kb and 136 kb region spanning the GLUC1.1A (SEQ ID NO: 53) and GLUC1.1D alleles (not shown), respectively, of *Gossypium hirsutum* was undertaken using 454 DNA sequencing (454 Life Sciences): Firstly BAC clones with genomic DNA spanning each GhGLUC1.1 allele were identified by hybridization using part of the GLUC1.1 gene as a probe against a FM BAC library. The BAC clones were isolated, confirmed by PCR and grouped into alleles. Selected BAC clones were sequenced to define neighboring genes facilitated by bioinformatics annotation software programs and EST searches (see FIG. **9**). The BAC sequence data also identified an additional molecular marker (CIR280) located on an adjacent gene (HAT) (see Table 6 and 7 for estimated position on chromosome A05 in the FM BC1 population).

Example 3

Analysis of the Biological Role of Glucanase in Fiber Strength

3.1. Determination of Link Between Inactive GbGLUC1.1A Enzyme and Fiber Strength

To determine if there is a link between the inactive GbGLUC1.1A enzyme and fiber strength, the impact of glucanase activity on fiber strength was analyzed by exogenous addition of a 1,3-beta-glucanase enzyme to fibers from *G. barbadense* (comprising a GLUC1.1A predicted to be inactive), as well as fibers from *G. hirsutum* (comprising a GLUC1.1A predicted to be active). It was expected that the strength of the *G. barbadense* fibers would significantly decrease, if there was indeed a link between the inactive GbGLUC1.1A enzyme and fiber strength.

Individual fibers were treated with a beta-1,3-D-glucanase from *Helix pomatia* (Fluka, 49103). 10 mg of fibers were incubated in 10 mM sodium acetate buffer (pH 5) and 500 µl of glucanase (1 mg/ml) was added. They were subjected to infiltration under vacuum for 10 minutes and overnight incubation at 37° C. The strength of individual cotton fibers was measured using a Favimat R device (Textechno) in a single fiber tensile test at 8 mm gauge length and a speed of 4 mm/min. The strength measure is recorded in force (cN). The results were statistically analyzed and are presented in Table 9 and FIG. **4**.

TABLE 9

Callose content (as measured by the green/blue fluorescence ratio of aniline blue stained fibers (ratio green/blue)) and strength (as measured by the breaking force (cN)) of untreated fibers (no GLUC) and fibers treated with glucanase (GLUC) from different *G. hirsutum* and *G. barbadense* varieties

| *Gossypium* species | Treatment | | Ratio green/blue | Force (cN) |
|---|---|---|---|---|
| *G. hirsutum* cv. FM966 (greenhouse) | No GLUC | Mean | 0.44 | 2.92 |
| | | SD | 0.04 | 1.92 |
| | GLUC | Mean | 0.43 | 3.11 |
| | | SD | 0.06 | 1.74 |
| *G. hirsutum* cv. FM966 (field US) | No GLUC | Mean | 0.51 | 5.50 |
| | | SD | 0.09 | 2.70 |
| | GLUC | Mean | 0.55 | 4.45 |
| | | SD | 0.10 | 2.03 |
| *G. hirsutum* cv. FM966 (field AU) | No GLUC | Mean | 0.52 | 4.33 |
| | | SD | 0.09 | 1.72 |
| | GLUC | Mean | 0.51 | 3.30 |
| | | SD | 0.14 | 1.43 |
| *G. hirsutum* cv. Coker312 (greenhouse) | No GLUC | Mean | 0.47 | 4.49 |
| | | SD | 0.02 | 2.45 |

TABLE 9-continued

Callose content (as measured by the green/blue fluorescence ratio of aniline blue stained fibers (ratio green/blue)) and strength (as measured by the breaking force (cN)) of untreated fibers (no GLUC) and fibers treated with glucanase (GLUC) from different *G. hirsutum* and *G. barbadense* varieties

| *Gossypium* species | Treatment | | Ratio green/blue | Force (cN) |
|---|---|---|---|---|
| | GLUC | Mean | 0.44 | 3.08 |
| | | SD | 0.06 | 1.63 |
| *G. barbadense* cv. PimaS7 (greenhouse) | No GLUC | Mean | 0.60 | 5.31 |
| | | SD | 0.05 | 2.26 |
| | GLUC | Mean | 0.49 | 2.76 |
| | | SD | 0.15 | 1.80 |
| *G. barbadense* cv. PimaY5 (field AU) | No GLUC | Mean | 0.61 | 5.19 |
| | | SD | 0.03 | 2.57 |
| | GLUC | Mean | 0.53 | 2.13 |
| | | SD | 0.04 | 1.20 |

A pronounced drop in strength was observed for Pima fibers treated with the glucanase and a less pronounced but still noticeable reduction in strength was observed for fibers from various *G. hirsutum* lines. In this respect, it is important to note that the extent of secondary cell wall formation and cellulose content contribute to fiber strength in *G. hirsutum*, while the stronger fibers of *G. barbadense* have a lower cellulose content than those of *G. hirsutum*. The complementation experiment thus indicated that the presence of the Gbgluc1.1A allele within the fiber strength locus contributes to the renowned strength of Pima fibers.

3.2. Determination of Link Between 1,3-Beta-D-Glucan Content and Fiber Strength

1,3-beta-D-glucans, including long chain 1,3-beta-D-glucans called callose, are the substrate for 1,3-beta-glucanase enzymes. Aniline blue is a dye specific for 1,3-beta-glucans. This dye was used to determine if fibers treated with 1,3-beta-glucanase and displaying a reduced fiber strength also displayed a reduced level of the 1,3-beta-glucan substrate in the cotton fiber walls.

A 0.05% solution of aniline blue in 0.067M $K_2HPO_4$ (pH 9) was used. The fibers were incubated for 15 minutes under vacuum. Under UV, callose deposits present an intense yellow-green fluorescence. Images are analyzed and the ratio Green/Blue is used as a measure for callose. The average value of 3 images was calculated.

As indicated in Table 9 and FIG. **5**, this staining technique showed that cotton fibers treated with the glucanase had a lower level of 1,3-beta-glucan and that elevated 1,3-beta-glucan levels were linked to enhanced fiber strength.

3.3. Statistical Analysis of Effect of Glucanase Treatment on Fiber Strength and Callose Content

The effect of the treatment (untreated minus treated) was statistically analyzed. The results are presented in Table 10.

TABLE 10

Statistical analysis of glucanase treatment (untreated minus treated) on callose content and strength of fibers from different *G. hirsutum* and *G. barbadense* varieties

| | Callose content (ratio G/B) | | Fiber strength (Force) | |
|---|---|---|---|---|
| | difference | p-value | difference | p-value |
| *G. hirsutum* cv. FM966 (greenhouse) | 0.01 | 0.882 | −0.18 | 0.618 |
| *G. hirsutum* cv. FM966 (field US) | −0.04 | 0.634 | 1.05 | 0.041* |
| *G. hirsutum* cv. FM966 (field AU) | 0.01 | 0.922 | 1.03 | 0.003* |

TABLE 10-continued

Statistical analysis of glucanase treatment (untreated minus treated) on callose content and strength of fibers from different *G. hirsutum* and *G. barbadense* varieties

| | Callose content (ratio G/B) | | Fiber strength (Force) | |
|---|---|---|---|---|
| | difference | p-value | difference | p-value |
| *G. hirsutum* cv. Coker312 (greenhouse) | 0.03 | 0.415 | 1.41 | 0.002* |
| *G. barbadense* cv. PimaS7 (greenhouse) | 0.11 | 0.278 | 2.55 | 0.000* |
| *G. barbadense* cv. PimaY5 (field AU) | 0.08 | 0.121 | 3.07 | 0.000* |

The correlations between the treatment and callose content as well as fiber strength were statistically analyzed. The results are presented in Table 11 for *G. hirsutum* and in Table 11 for *G. barbadense*.

TABLE 11

Statistical analysis of correlations between glucanase treatment of fibers of *G. hirsutum*, their callose content and their strength

| | | Glucanase treatment | Callose content (ratio G/B) | Fiber strength (Force) |
|---|---|---|---|---|
| Glucanase treatment | Correlation | 1.00 | −0.03 | −0.48 |
| | Sig. (2-tailed) | | 0.944 | 0.233 |
| Callose content (ratio G/B) | Correlation | −0.03 | 1.00 | 0.66 |
| | Sig. (2-tailed) | 0.944 | | 0.075 |
| Fiber strength (Force) | Correlation | −0.48 | 0.66 | 1.00 |
| | Sig. (2-tailed) | 0.233 | 0.075 | |

TABLE 12

Statistical analysis of correlations between glucanase treatment of fibers of *G. barbadense*, their callose content and their strength

| | | Glucanase treatment | Callose content (ratio G/B) | Fiber strength (Force) |
|---|---|---|---|---|
| Glucanase treatment | Correlation | 1.00 | −0.96 | −0.99 |
| | Sig. (2-tailed) | | 0.044* | 0.013* |
| Callose content (ratio G/B) | Correlation | −0.96 | 1.00 | 0.90 |
| | Sig. (2-tailed) | 0.044* | | 0.103 |
| Fiber strength (Force) | Correlation | −0.99 | 0.90 | 1.00 |
| | Sig. (2-tailed) | 0.013* | 0.103 | |

In summary, cotton fibers with a higher 1,3-beta-glucan content displayed higher fiber strength and reduction in 1,3-beta-glucan content by exogenously supplied 1,3-beta-glucanase enzyme significantly reduced fiber strength and callose content in *G. barbadense*, indicating that 1,3-beta-glucan or callose has a specific role in cotton fiber strength which can be modulated by enzymes such as GLUC1.1.

## Example 4

### Identification of GLUCL1A Alleles in Different Cotton Species

GLUC1.1 sequences were isolated from six different *Gossypium hirsutum* varieties (Guazuncho; DP16; Cooker 312

(C312); Fiber Max 966 (FM966); Acala SJ2; Acala Maxxa), from five different *Gossypium barbadense* varieties (PimaS7; Tanguis LMW 1737-60; Tanguis CN(C.P.R.) 712-60; Sea Island Tipless; VH8), from *Gossypium herbacium, Gossypium tomentosum, Gossypium darwinii, Gossypium arboreum, Gossypium raimondii, Gossypium kirkii, Gossypium longicalyx*, and *Gossypium mustelinum*

Based on the GhGLUC1.1A and D nucleotide sequences described in WO2008/083969 (SEQ ID NO: 1 and 7, respectively), primer pairs (forward primer SE077 (SEQ ID NO: 33) and G1.1-f1-F1 (SEQ ID NO: 65) en reverse primer SE078 (SEQ ID NO: 34) or forward primer SE002 (SEQ ID NO: 35) en reverse primer SE003 (SEQ ID NO: 36)) were designed to amplify full-length or partial, respectively, genomic DNA fragments. The reaction mix used contained: DNA (200 ng/μl genomic DNA), 1 μl forward primer (10 pM), 1 μl reverse primer (10 pM), 4 μl 5× High Fidelity buffer, 0.2 μl Phusion enzyme (Finnzymes), 0.4 μl dNTP's (10 mM), 11.4 μl water (MilliQ). The PCR protocol used was as follows: 1 min at 98° C.; 30 times: 10 sec at 98° C. (denaturation), 30 sec at 56° C. (annealing), 1 min at 72° C. (elongation); 30 sec at 58° C.; 10 min at 72° C.; 4° C.

GLUC1.1A sequences from all *G. barbadense* lines tested and from *Gossypium darwinii* display a single nucleotide substitution (c to t at position 712 of SEQ ID NO: 5 and at position 470 of SEQ ID NO: 17 or at position 761 of SEQ ID NO: 56, respectively; see also GLUC1.1A-SNP5 in Table 13) resulting in a premature stop codon (cga to tga) in their sequences (FIG. **6**; since the GLUC1.1 sequences from the different *Gossypium hirsutum* varieties and the different *Gossypium barbadense* varieties, respectively, were identical to each other, only the GLUC1.1 sequences of the FM966 and PimaS7 variety, respectively, were included in the alignment). The GLUC1.1A sequence from *G. arboreum* displayed a single nucleotide deletion (deletion of c nucleotide between position 327 and 328 of SEQ ID NO: 21) also resulting in a premature stop codon (tga at position 373-375 of SEQ ID NO: 21) further downstream in its sequence (FIG. **6**). The premature stop codons in the GLUC1.1A sequences from *G. barbadense*, from *Gossypium darwinii* and from *G. arboreum* resulted in a predicted truncated GLUC1.1A protein sequence (FIG. **7**; GLUC1.1A protein of 179 (SEQ ID NO: 6), of 179 (SEQ ID NO: 57), and of 78 (SEQ ID NO: 22) amino acids, respectively), while the GLUC1.1A sequences from all other *Gossypium* species tested did not display premature stop codons and are predicted to produce a complete GLUC1.1 protein (FIGS. **6** and **7**).

As indicated above, *G. barbadense* is commercially recognized for its superior fiber quality, particularly for fiber strength, length and fineness. *G. darwinii* is the closest relative of *G. barbadense* and some even consider it as a variety of *G. barbadense* rather than a separate species. However, *G. darwinii* produces sparse, non-spinnable, khaki or brown fiber, usually less than 1.3 cm in length (see e.g. Wendel and Percy, 1990, Bioch. Systematics And Ecology 18 (7/8): 517-528). As the fibers from *G. darwinii* are not commercially used, little information is available about its commercially relevant fiber qualities, such as fiber strength.

## Example 5

### Genotyping of GLUCL1 Genes in Commercial Germplasm

The genotype of GLUC1.1A and GLUC1.1D genes was determined in commercially available germplasm by determining the genotype of GLUC1.1A-SNP3, 5 and 6 and GLUC1.1D-SNP1 (as indicated in FIG. **6** and Table 13) in a total of 73 *G. hirsutum* varieties, one *G. barbadense* variety, 2 *G. arboreum* varieties, one *G. herbaceum* variety, and one *G. mustilinum* variety using Illumina GoldenGate SNP Genotyping and BeadArray technology as prescribed by the manufacturer. Briefly, a GoldenGate Genotyping assay uses allele-specific extension and ligation for genotype calling using a discriminatory DNA polymerase and ligase (Illumina).

TABLE 13

Position and genotype of GLUC1.1D-SNP1 and GLUC1.1A-SNP2, 3, 5, 6, 7 and 8 in GLUC1.1D and A genes, respectively of different *Gossypium* species (*G. h.*: *G. hirsutum*, *G. b.*: *G. barbadense*, *G. t.*: *G. tomentosum*; *G. d.*: *G. darwinii*; *G. m.*: *G. mustilinum*; *G. a.*: *G. arboreum* *G. he.*: *G. herbaceum* *G. r.*: *G. raimondii*)

| GLUC1.1A | | | | | | | |
|---|---|---|---|---|---|---|---|
| *G.* sp.:<br>SEQ ID: | *G. h.*<br>1 | *G. b.*<br>5 | *G. t.*<br>15 | *G. d.*<br>56/17 | *G. m.*<br>19 | *G. a.*<br>21 | *G. he.*<br>23 |
| SNP7 between | 2674-2676 | 327-329 | 85-87 | 376-378/85-87 | 85-87 | 327-328 | 327-329 |
| | C | C | C | C | C | — | C |
| SNP2 between | 2765-2766 | 418-428 | 176-177 | 467-477/176-186 | 176-177 | 417-418 | 418-419 |
| | — | CTCAT CAAA | — | CTCAT CAAA | — | — | — |
| SNP3 | 2911 | 573 | 322 | 622/331 | 322 | 563 | 564 |
| | G | C | G | C | C | C | C |
| SNP5 | 3050 | 712 | 461 | 761/470 | 461 | 702 | 703 |
| | C | T | C | T | C | C | C |
| SNP8 | 3170 | 832 | 581 | 881/590 | 581 | 821 | 823 |
| | G | C | G | G | G | G | G |
| SNP6 | 3202 | 864 | 613 | 913/622 | 613 | 854 | 855 |
| | G | A | G | A | G | G | G |

TABLE 13-continued

Position and genotype of GLUC1.1D-SNP1 and GLUC1.1A-SNP2, 3, 5, 6, 7 and 8 in GLUC1.1D and A genes, respectively of different *Gossypium* species (*G. h.*: *G. hirsutum*, *G. b.*: *G. barbadense*, *G. t.*: *G. tomentosum*; *G. d.*: *G. darwinii*; *G. m.*: *G. mustilinum*; *G. a.*: *G. arboreum* *G. he.*: *G. herbaceum* *G. r.*: *G. raimondii*)

| GLUC1.1D | | | | | | |
|---|---|---|---|---|---|---|
| *G.* sp.: SEQ ID: | *G. h.* 7 | *G. b.* 11 | *G. t.* 25 | *G. d.* 58/27 | *G. m.* 29 | *G. r.* 31 |
| SNP1 | 3614 C | 304 T | 80 C | 352/80 T | 80 C | 80 C |

The results confirmed that the genotypes of GLUC1.1A-SNP3, 5 and 6 and GLUC1.1D-SNP1 in the different analysed *Gossypium* species and varieties were as indicated in FIG. **6** and Table 13. In particular, genotyping of GLUC1.1A-SNP5 in the different *Gossypium* species and varieties indicated that all analysed *Gossypium* species and varieties different from *G. barbadense* comprise the cga codon found in GLUC1.1A of *Gossypium hirsutum* instead of the tga stop codon found in gluc1.1A of *Gossypium barbadense* Pima S7.

## Example 6

### Detection of GLUCL1 Allele Encoding an Inactive GLUC1.1 Protein in *Gossypium* Plants and/or Transfer of GLUCL1 Allele Encoding an Inactive GLUC1.1 Protein into *Gossypium* Lines Comprising a Corresponding GLUCL1 Allele Encoding an Active GLUC1.1 Protein

A GLUC1.1 allele encoding an inactive GLUC1.1 enzyme, such as a Gbgluc1.1A allele, Gdgluc1.1A allele or Gagluc1.1A allele, is transferred into cotton lines comprising a corresponding GLUC1.1 allele encoding an active GLUC1.1 enzyme, such as *Gossypium hirsutum* breeding lines, by the following method:

A plant containing a GLUC1.1 allele encoding an inactive GLUC1.1 enzyme, such as a *Gossypium barbadense* plant, a *Gossypium darwinii* plant or a *Gossypium arboreum* plant containing a GLUC1.1A allele encoding an inactive GLUC1.1A enzyme, or a mutagenized *Gossypium hirsutum* plant containing a mutant GLUC1.1 allele encoding an inactive GLUC1.1 enzyme (donor plant), is crossed with a plant containing a corresponding GLUC1.1 allele encoding an active GLUC1.1 enzyme, such as a *Gossypium hirsutum* plant containing a GLUC1.1A allele encoding an active GLUC1.1A enzyme (recurrent parent). The following introgression scheme is used (the GLUC1.1 allele encoding an inactive GLUC1.1 enzyme is abbreviated to gluc while the GLUC1.1 allele encoding an active GLUC1.1 enzyme is depicted as GLUC):

Initial cross: gluc/gluc (donor)×GLUC/GLUC (recurrent parent)

F1 plant: GLUC/gluc

BC1 cross: GLUC/gluc (F1)×GLUC/GLUC (recurrent parent)

BC1 plants: 50% GLUC/gluc and 50% GLUC/GLUC

The 50% GLUC/gluc are selected using a specific assay (e.g. PCR, TaqMan™, Invader™, and the like; see also below) for the gluc1.1 allele.

BC2 cross: GLUC/gluc (BC1)×GLUC/GLUC (recurrent parent)

BC2 plants: 50% GLUC/gluc and 50% GLUC/GLUC

The 50% GLUC/gluc are selected using a specific assay (e.g. PCR, TaqMan™, Invader™, and the like; see also below) for the gluc1.1 allele.

Backcrossing is repeated until BC4 to BC5 (e.g. if the donor plant is a *Gossypium barbadense* plant and the recurrent parent is a *Gossypium hirsutum* plant) or until BC3 (e.g. if the donor plant and the recurrent parent are *Gossypium hirsutum* plants)

BC3-5 plants: 50% GLUC/gluc and 50% GLUC/GLUC

The 50% GLUC/gluc are selected using a specific assay (e.g. PCR, TaqMan™, Invader™, and the like; see also below) for the gluc1.1 allele.

To reduce the number of backcrossings (e.g. until BC2 if the donor plant and the recurrent parent are *Gossypium hirsutum* plants, or until BC3 to BC4 if the donor plant is a *Gossypium barbadense* plant and the recurrent parent is a *Gossypium hirsutum* plant), molecular markers can be used in each generation that are specific for the genetic background of the recurrent parent.

BC3-5 S1 cross: GLUC/gluc×GLUC/gluc

BC3-5 S1 plants: 25% GLUC/GLUC and 50% GLUC/gluc and 25% gluc/gluc

Plants containing the gluc1.1 allele are selected using molecular markers for the gluc1.1 allele. Individual BC3-5 S1 plants that are homozygous for the gluc1.1 allele (gluc/gluc) are selected using molecular markers for the gluc1.1 and GLUC1.1 alleles. These plants are then used for fiber production.

Molecular markers which can be used to detect a specific gluc1.1 or GLUC1.1 allele or to discriminate between a specific gluc1.1 and GLUC1.1 allele are, for example, single nucleotide polymorphisms (SNPs) or polymorphic nucleotide sequences:

As an example, SNPs and polymorphic nucleotide sequences which can be used to discriminate between the Gbgluc1.1A or Gdgluc1.1A allele and the GhGLUC1.1A allele and between the GbGLUC1.1D or Gdgluc1.1D allele and the GhGLUC1.1D allele or to detect their presence in DNA samples or plants, are SNPs indicated as GLUC1.1A-SNP3, 5 and 6 in FIG. **6** and Table 13 and the polymorphic nucleotide sequence indicated as GLUC1.1A-SNP2 in FIG. **6** and Table 13 and the SNP indicated as GLUC1.1D-SNP1 in FIG. **6** and Table 13, respectively.

In particular, a SNP which can be used to discriminate between the Gbgluc1.1A or Gdgluc1.1A allele that comprises a premature tga STOP codon and the corresponding GhGLUC1.1A allele that comprises a cga codon instead, is the SNP indicated as GLUC1.1A-SNP5 in FIG. **6** and Table 13.

The genotype of such SNPs and polymorphic nucleotide sequences can be determined, for example, using a PCR assay.

As an example, PCR assays were developed to determine the genotype of the SNP indicated as GLUC1.1D-SNP1 in FIG. **6** and Table 13 and of the polymorphic nucleotide sequence indicated as GLUC1.1A-SNP2 in FIG. **6** and Table 13 of plants of the BC1 populations described in Example 1 in order to map the GLUC1.1D and A genes of *G. hirsutum* and *barbadense*, respectively. More specifically, following PCR assay was developed to discriminate between the Gbgluc1.1A allele and the GhGLUC1.1A allele based on the genotype of the SNP indicated as GLUC1.1A-SNP2 in FIG. **6** and Table 13:

Primers:

```
Forward:    5' TAT CCC TCT CGA TGA GTA CGA C 3'
            (p1.3GlucaAf - SEQ ID NO: 37)

Reverse:    5'CCC AAT GAT GAT GAA CCT GAA TTG3'
            (p1.3GlucaAr - SEQ ID NO: 38)
```

Amplicon size: 134 bps for *G. hirsutum* and 143 bps for *G. barbadense*.

PCR conditions: 50 gDNA (20 ng/μl)+15 μl PCR mix (PCR mix: 2 μl 10×Taq PCR buffer, 1 μl labeled p1.3GlucaAf (100 pmol/μl), 0.2 μl p1.3GlucaAr (100 pmol/μl), 0.25 μl dNTPs (20 mM), 0.5 μl $MgCl_2$ (50 mM), 0.2 μl Taq polymerase, 10.85 μl MiliQ)

Labeling of forward primer: 0.1 μl 10×T4 kinase buffer, 0.2 μl p1.3GlucaAf (100 pmol/μl), 0.01 μl T4 kinase, 0.1 μl $P^{33}\gamma$ ATP, 0.59 μl MilliQ=1 μl; 1 h at 37° C. and 10 min at 65° C.

PCR profile: 5 min at 95° C.; 35 times: 45 s at 95° C., 45 s at 58° C., 1 min at 72° C.; 10 min at 72° C.

Gel analysis: PCR fragments are separated on 4.5% denaturing acrylamide gels



Overnight exposure of gel to BIOMAX MR films

Alternatively, the genotype of such SNPs can be determined, for example, using Illumina GoldenGate SNP Genotyping as indicated in Example 5 for the SNPs indicated as GLUC1.1A-SNP3, 5 and 6 and GLUC1.1D-SNP1 in FIG. **6** and Table 13.

Alternatively, the genotype of such SNPs and polymorphic nucleotide sequences can be determined by direct sequencing by standard sequencing techniques known in the art to determine the complete GLUC1.1 nucleotide sequence present in a plant followed by analysis of the obtained sequence, e.g., by alignment with the GLUC1.1 sequences described herein (see, e.g., FIGS. **6** and **7**).

Alternatively, the genotype of such SNPs and polymorphic nucleotide sequences can be determined by a Taqman assay. The TaqMan assay procedure and interpretation of the data are performed as prescribed by the manufacturer (Applied Biosystems). Briefly, a probe specific for a specific variant of a polymorphic site in a GLUC1.1 gene binds the template DNA if this specific variant is present. The probe has a fluorescent reporter or fluorophore, such as 6-carboxyfluorescein (acronym: FAM) and VIC (a proprietary dye from Applied Biosystems), attached to its 5' end and a quencher (e.g., tetramethylrhodamine, acronym: TAMRA, of dihydrocyclopyrroloindole tripeptide "minor groove binder", acronym: MGB) attached to its 3' end. The close proximity between fluorophore and quencher attached to the probe inhibits fluorescence from the fluorophore. During a PCR with two primers capable of amplifying a DNA fragment comprising the polymorphic site, the 5' to 3' exonuclease activity of the Taq polymerase degrades that proportion of the probe that has annealed to the template as DNA synthesis commences. Degradation of the probe releases the fluorophore from it and breaks the close proximity to the quencher, thus relieving the quenching effect and allowing fluorescence of the fluorophore. Hence, fluorescence detected in the real-time PCR thermal cycler is directly proportional to the fluorophore released and the amount of DNA template present in the PCR. The following discriminating Taqman probes and primers were thus developed to discriminate different variants of GLUC1.1A-SNP3 and GLUC1.1A-SNP5 (see FIG. **6** and Table 13):

TABLE 14a

| GLUC1.1A - SNP3 of Probes | | |
|---|---|---|
| Gbgluc1.1A | 5' FAM- AACTCGCTCGCCTCA 3' | (SEQ ID NO: 39) |
| GhGLUC1.1A | 5' VIC-AACTCGCTGGCCTCA 3' | (SEQ ID NO: 40) |
| Forward primer | 5' CCTGGTGCCATGAACAACATAATG 3' | (SEQ ID NO: 41) |
| reverse primer | 5' CGTCGTGCCTAGCCCAAA 3' | (SEQ ID NO: 42) |

TABLE 14b

| GLUC1.1A - SNP5 of Probes | | |
|---|---|---|
| Gbgluc1.1A | 5' FAM- ATCCTGTCAAACCAG 3' | (SEQ ID NO: 60) |
| GhGLUC1.1A | 5' VIC-ATCCTGTCAAACCAG 3' | (SEQ ID NO: 61) |
| Forward primer | 5' GCTTTTGGAAGCGATATAACATCGA 3' | (SEQ ID NO: 62) |
| reverse primer | 5' GGCATAGGCAAAATAAGGGTACACA 3' | (SEQ ID NO: 63) |

Probes specific for polymorphic sites in the Gbgluc1.1A or corresponding GhGLUC1.1A target gene, such as the probes specific for GLUC1.1A-SNP3 of Gbgluc1.1A and GhGLUC1.1A indicated as "5' FAM-AACTCGCTCGC-CTCA 3" and "5' VIC-AACTCGCTGGCCTCA 3', respectively, in Table 14a, and forward and reverse primers that are capable of amplifying a fragment comprising the polymorphic site and that can thus be used in combination with them are indicated in Table 14a. Generally, each probe set consists of two probes each specific for one variant of the polymorphic site in the GLUC1.1 target gene which comprises the variant nucleotide (e.g., the underlined nucleotide in Table 14) or variant nucleotide sequence (e.g. the probe with SEQ ID NO: 39 is specific for GLUC1.1A-SNP3 of Gbgluc1.1A and the probe with SEQ ID NO: 40 is specific for GLUC1.1A-SNP3 of GhGLUC1.1A) and a set of two primers that are capable of amplifying a fragment comprising the polymorphic site (e.g. the primer with SEQ ID NO: 41 is specific for a nucleotide sequence upstream of GLUC1.1A-SNP3 and the primer with SEQ ID NO: 42 is specific for a nucleotide sequence downstream of GLUC1.1A-SNP3, such that the use of both primers results in the amplification of a DNA fragment comprising GLUC1.1A-SNP3).

Alternatively, the genotype of such SNPs and polymorphic nucleotide sequences can be determined by Invader™ technology (Third Wave Agbio).

## Example 7

### Comparison of Expression of GLUCL1A and GLUCL1D During Fiber Growth and Development in *Gossypium barbadense* and in *Gossypium hirsutum*

Expression of GLUC1.1A and GLUC1.1D during fiber growth and development was analyzed for *G. barbadense* and compared with the expression of GLUC1.1A and GLUC1.1D during fiber growth and development of *G. hirsutum* as described in WO2008/083969.

DNA from a cDNA library of *G. barbadense* created from fiber cells and seed at 0 and 5 DPA and from fiber cells at 10, 15, 20, 25, 30 and 40 DPA was extracted, the concentration was equalized and a PCR amplification was performed using primers SE002 (SEQ ID NO: 35) and SE003 (SEQ ID NO: 36). The PCR reaction mix used contained: 1 μl template DNA (200 ng/μl), 5 μl 5× GreenGoTaq buffer, 0.75 μl SE002 (10 μM), 0.75 μl SE003 (10 μM), 0.5 μl dNTP's (20 mM), 0.25 μl GoTaq polymerase, 16.75 μl MilliQ water (total of 25 μl). The PCR conditions used were as follows: 5 min at 95° C.; 5 times: 1 min at 95° C., 1 min at 58° C., 2 min at 72° C.; 25 times: 30 s at 92° C., 30 s at 58° C., 1 min at 72° C.; 10 min at 72° C., cooldown to 4° C. The expected length of the PCR product is 655 bp. After PCR amplification, the PCR fragment is digested with AlwI digest (3 h incubation at 37° C.) using 10 μl template; 1 μl AlwI enzyme; 2 μl NEB 4 restriction buffer; 7 μl MQ water. The resulting fragments are analysed on 1.5% TAE gel stained with EtBr. The expected band sizes for the A subgenome allele specific PCR fragment are: 479 bp, 118 bp and 59 bp (not visible in FIG. **8**). The expected band sizes for the D subgenome allele specific PCR fragment are: 538 bp and 118 bp.

FIG. **8**, lanes 2 to 9, represent GbGLUC1.1A and D expression at 0, 5, 10, 15, 20, 25, and 40 DPA. Differences in band intensities in FIG. **8** correspond to relative differences in expression. A negative (no template; NTC; FIG. **8**, lane 10) and a positive control (genomic DNA from Pima S7; FIG. **8**, lane 11) were included. The expression profile of the GhGLUC1.1A and D and GbGLUC1.1A and D genes can be summarized as follows:

| | Days post anthesis (DPA): | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 40 |
| GhGLUC1.1 | — | — | — | D | D | ND | A & D | A & D |
| GbGLUC1.1 | — | — | — | A & D | A & D | A & D | A & D | A & D |

Thus while the expression of GLUC1.1A in *G. hirsutum* starts only at 30 DPA, GLUC1.1A in *G. barbadense* is expressed from 15 DPA on. However, as indicated above, the GbGLUC1.1A gene is predicted to encode a non-functional GLUC1.1A protein.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 65

<210> SEQ ID NO 1
<211> LENGTH: 6009
<212> TYPE: DNA
<213> ORGANISM: Gossypium hirsutum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2410)..(2443)
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2556)..(3496)

<400> SEQUENCE: 1

```
cgcggcatat aattttatgt gtgtaatttg ttgggttaat tacttaaaat agtatatttt     60

taattgctgt aattaatgta agataatttt tattatttga atcattgcac aaaattaaaa    120

tagaataatt tatttaacaa ttcaaatata ataataatcc aaattataat tatagtattt    180

ttacaatatt caatatacaa tatagtttta cttcatacaa ttaatataaa aaaatattat    240

tcaaaataat aactaataaa cataattacc atatattaat tattttgata tttcgaacat    300

aacgctaata aaaaatttcc taatcattat taaatcattt gtataaacta taaagaaatt    360

gatatattgt aaattaaact ttattcattt tttttcttaa tactcaataa attaatcata    420

ataactcata aataatatat aattaaaata atcataacat ggtagattat ataaataggg    480

ggcgaatcta gggagctggc atgaccccta aaatagaatt ttctattttg acctatcaaa    540

atttttaaaa ttttaaatta gtaaaggtaa atttgtactt tgacctctta aaatgataaa    600

attttacttt aatcctttaa aatttacatt tttactatca taaaaattac aatttgattt    660

tgcccctaaa atttttttct agcttagccc tgtatataaa tatattattt ataattttta    720

tatttaaaat ataaagtttt taattataca aataattaaa atctgatatt taaaactaaa    780

gtaatttctt ttttcttttt actttttttt aattgcaaca taatggttta aatatctata    840

taacgtatga agtaatttga tataaatttt attttaattt attattatat aaattcattt    900

agtaaaaact tttaatagaa tcaaaatttt tatttgtaaa ttcgataact tttcttatca    960

agtatatttg tgagaaccaa atatttagta aaattaatat tcttatttat aaatatgata   1020

aatcttataa aaaaatattt aaaatgaaaa aaattgtaca aatattataa aaaaatattt   1080

aaaatgaaaa acattgtaca aaggctatat aagaagttca aaagtttctt cgaccatgta   1140

ctcttataga gattatagat agattataaa actatatgta gtttctctta acttttaaat   1200

aagaggataa atgtatttta atgtactcaa acttatatat ttttatattg acaataatat   1260

caatatcaac ctaattaaga ttcattctaa cattaatgtt gaagattttt aataaaagaa   1320

aaggttaata aattaattag aacacaaaca aacacaaatt taagtggtat gtaaggtcct   1380

tgacccaaag gaaaaatttg ttacgtcgat taaattataa attaatttaa agtaaaatta   1440

cattttaacc taaaaaaaga gaaaagtata tctaatttct tcgaaaatgg aaagaaaatt   1500

ataaatttat ggcatttcta aaaaaattct gaattcgcta ctaaaagatg aaattataaa   1560

atccgaagca ttaccagaag atggatcacc aaatcacaaa caatcaatga aaagtaatga   1620

taattaattg aaagtgagca tttaattttg atagccatat acttcctgct gaatttatag   1680

gttctcatta atgcaattaa attatattcg acaccttttg aatgaaataa aatgacacaa   1740

gaggaaagac ggttcatcta ttttttcttt caatcgccca tcaaaatacc aaaaatgtaa   1800

ctacatgcaa aaaatcaaat atgaaaaata ttcatatttt gatattttaa tatattgtgt   1860

gttcaaaacg taaatgtatt gaaaaattat gatggtgttg ttgctgtatg tccataaaat   1920
```

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tcaatgtact cacatttatc aaatgtatac tttgagagaa gttattttga taatactcaa   1980

gtttttttta tagatgggaa aattttttaa attatttttt gattttgatg aaatgtatat   2040

ataaatttta attcgataca tataaatata tatgtaaatt ttaaatttaa atttaataat   2100

atacaattaa gaaaataatt tataaatatt ttccgattaa aaataaatct ggaaagaaga   2160

aatgtcaaca ctttttcatt aaatacaatt aggatgggac acgatacctt catgcattga   2220

tatctcaggt ggtccaaaaa ctcggaatcc tttttgaaaa aaaacttcca gagagagtat   2280

ataaatccag cagtaggcac aagaaacgag caccagttat tgactttcct ttgtaaaaaa   2340

aaaaagtgct gagatcaaga aatatagtga aatatgggtc caagattttc tgggttttta   2400

atctaagca atg ctg ttt tta act caa ctc ctc tct cta aca g             2443
          Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr
          1               5                   10

gtaaaacaaa cttctctaca gtgattttac agtaaatatg gctttgaaaa atatacaaca   2503

aaacatttat cttcaatcca ttttaattac tgatctacta tatatgttgc ag at  ggc   2560

Asp Gly
cgt gat att ggt gtt tgc tat ggt ttg aac ggc aac aat ctt cca tct     2608
Arg Asp Ile Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser
    15                  20                  25

cca gga gat gtt att aat ctt ttc aaa act agt ggc ata aac aat atc     2656
Pro Gly Asp Val Ile Asn Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile
30                  35                  40                  45

agg ctc tac cag cct tac cct gaa gtg ctc gaa gca gca agg gga tcg     2704
Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser
                50                  55                  60

gga ata tcc ctc tcg atg agt acg aca aac gag gac ata caa agc ctc     2752
Gly Ile Ser Leu Ser Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu
            65                  70                  75

gca acg gat caa agt gca gcc gat gca tgg gtt aac acc aac atc gtc     2800
Ala Thr Asp Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val
        80                  85                  90

cct tat aag gaa gat gtt caa ttc agg ttc atc atc att ggg aat gaa     2848
Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu
    95                  100                 105

gcc att cca gga cag tca agc tct tac att cct ggt gcc atg aac aac     2896
Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn
110                 115                 120                 125

ata atg aac tcg ctg gcc tca ttt ggg cta ggc acg acg aag gtt acg     2944
Ile Met Asn Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr
                130                 135                 140

acc gtg gtc ccg atg aat gcc cta agt acc tcg tac cct cct tca gac     2992
Thr Val Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp
            145                 150                 155

ggc gct ttt gga agc gat ata aca tcg atc atg act agt atc atg gcc     3040
Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala
        160                 165                 170

att ctg gtt cga cag gat tcg ccc ctc ctg atc aat gtg tac cct tat     3088
Ile Leu Val Arg Gln Asp Ser Pro Leu Leu Ile Asn Val Tyr Pro Tyr
    175                 180                 185

ttt gcc tat gcc tca gac ccc act cat att tcc ctc aac tac gcc ttg     3136
Phe Ala Tyr Ala Ser Asp Pro Thr His Ile Ser Leu Asn Tyr Ala Leu
190                 195                 200                 205

ttc acc tcg acc gca ccg gtg gtg gtc gac caa ggc ttg gaa tac tac     3184
Phe Thr Ser Thr Ala Pro Val Val Val Asp Gln Gly Leu Glu Tyr Tyr
                210                 215                 220

aac ctc ttt gac ggc atg gtc gat gct ttc aat gcc gcc cta gat aag     3232
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Asn Leu Phe Asp Gly Met Val Asp Ala Phe Asn Ala Ala Leu Asp Lys
            225                 230                 235

atc ggc ttc ggc caa att act ctc att gta gcc gaa act gga tgg ccg      3280
Ile Gly Phe Gly Gln Ile Thr Leu Ile Val Ala Glu Thr Gly Trp Pro
        240                 245                 250

acc gcc ggt aac gag cct tac acg agt gtc gcg aac gct caa act tat      3328
Thr Ala Gly Asn Glu Pro Tyr Thr Ser Val Ala Asn Ala Gln Thr Tyr
    255                 260                 265

aac aag aac ttg ttg aat cat gtg acg cag aaa ggg act ccg aaa aga      3376
Asn Lys Asn Leu Leu Asn His Val Thr Gln Lys Gly Thr Pro Lys Arg
270                 275                 280                 285

cct gaa tat ata atg ccg acg ttt ttc ttc gag atg ttc aac gag aac      3424
Pro Glu Tyr Ile Met Pro Thr Phe Phe Phe Glu Met Phe Asn Glu Asn
                290                 295                 300

ttg aag caa ccc aca gtt gag cag aat ttc gga ttc ttc ttc ccc aat      3472
Leu Lys Gln Pro Thr Val Glu Gln Asn Phe Gly Phe Phe Phe Pro Asn
            305                 310                 315

atg aac cct gtt tat cca ttt tgg tgaacttgaa atgttattgt tggctattta     3526
Met Asn Pro Val Tyr Pro Phe Trp
        320                 325

aatcttttgc cagagacgct tcatatagtt tctgcatatt ttgaaagtgg aaaatcaatc    3586

taaatataaa taagttttat ttgttgtttt ttaattaaat aaaattttaa atattttaaa    3646

aacatcttta ttggtaatta aatattaaat aaaaagttta atattcaaat tttatcaatt    3706

caaaaataaa ataaaaatat attaaattta tttttacgaa taaattgatt ttctattaat    3766

gcagatttta aataatttga tataaatttt caattcaaca atagtaattt tgatcacatc    3826

aaaggagaaa gggaaagatt taactttaat tggtgaccta atataacacg ttgaaaacgg    3886

agttcccaat aaggcaaaat gacttgtaat gacgaaagag atgtccaagt gaaatctgct    3946

ttaaagtgaa agaagcataa aaggataact aaataactca tgatctaaat tgaagttcta    4006

taaaatgcaa ctttcatcta gaaacaaggt atgtcttaaa tgatgtttta tgaatttgtc    4066

ttaattgggt tttatgcaat gaattcatgg atagcacatc tctaattata cgttgctggt    4126

ttatatgaga gtggtgcaga agttaattgt gctttaaata cttgcttagt gtttatgaaa    4186

tttgaaaagt gttatatact tataataaaa ataattcgat tcggaatcca attcagggtt    4246

cgactcaata taataaaatt ttacagatat cttgaagggg atcttcttct tctctacttc    4306

tcgagcagtg ttatatattt acaataaaga taactcaatt cgagatccga cctaatataa    4366

taaaattcta cagacatatc aaagagggag atcttcttct tccctacatc ttgaccttct    4426

tgatcaaaat gaccttcctt atatttttac atacgttgat tatatgaatc aaaagaaaga    4486

taccaaaaag tttttaaaaa taaacaacgg ggttcttatg tagagatgct tatgggccgg    4546

gccggactca actaaaaatt taggcacatt cattgggccc aggtcgggcc taacccaaaa    4606

atgggcctaa aattttgccc aagcttgact caaataaaaa tgctaaaatt cgggcctgac    4666

cccgtattaa ttttatatta ttttatataa cttttaaata tatataatat ataaaaaata    4726

ctaaaaaaat taaaataaat atttcccaac taaactaaaa ttattaagaa aaataattca    4786

tattagcgta taaattggaa attgaccaaa attaaaatta ttgtatagtt aatctatatt    4846

aaaaggacat gtaattaaaa accattaaaa ctattataca ataaattaaa tcttcattgt    4906

atacatagaa aggcattaat aattaaaaaa ctatattaag atataaacta aattcaaaat    4966

tattaaaaac aagaactaaa taaaaaagca attgaaaatt acgaattaat gttaaaatca    5026

aatgttaaaa tcaagggact taaataaaaa tatcccaaaa tacaaaacat tagcttcctt    5086
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tcccatccac gtgaatgcaa agtttacatg gtgtttccta gtgtttgtgc gactccaacc   5146

ttttatttac ctcttttttt ctttatttga acaattattt gataatgatt agaattttgg   5206

gattgttgct catcgtacgt gcaacactta aaatcactat gatttttcat aatttatata   5266

acctatatcg ttttggaaat taattttatt ttttatatta ttttaataaa aataccatct   5326

accttttta atttatgatc cctttcatat ttaaaaattc aaattgacaa ttgtctaact   5386

aaacaccgtc acactccaat aagattgtaa tttcctccat cttgatatta cactcaaaag   5446

catgttgcca acaaacaaat caactagcct ttttctacca ctattcatca tcttcttaag   5506

agtgtgttta tgtcatgtgc cgagatttta ggtatggtca cgttgtggct ttaaactcaa   5566

atctattgcc catgagtcta agttagcctc cgatcctcac taaagagagg cttggcacac   5626

tttacctagc caagtacaca aggaatagag ctattagaaa gcattaaaga gttaggagaa   5686

tgtggaagtg tttttattac tcaaagctaa cttggataca aataaaggag ggagcctctc   5746

ctttaggcaa gcttcttttg atctgatggt tacaattaat ctcgaatagg aggggtcaaa   5806

cttctcactc agtttcatat tatctcttgg tgcttggttg gcctccgcct tgagacaact   5866

ttagataaca cctagtctta acacttttag cttcacattg tacgcatcct tcattactca   5926

aatgccacaa agcctcctta cttaaggctc ttggtcgctc ccactacctt cggctttaga   5986

ctcatctaag atcttcccaa tcg                                           6009


&lt;210&gt; SEQ ID NO 2
&lt;211&gt; LENGTH: 325
&lt;212&gt; TYPE: PRT
&lt;213&gt; ORGANISM: Gossypium hirsutum

&lt;400&gt; SEQUENCE: 2

Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile
1               5                   10                  15

Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp
            20                  25                  30

Val Ile Asn Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr
        35                  40                  45

Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser
    50                  55                  60

Leu Ser Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp
65                  70                  75                  80

Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys
                85                  90                  95

Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu Ala Ile Pro
            100                 105                 110

Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn Ile Met Asn
        115                 120                 125

Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val
    130                 135                 140

Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe
145                 150                 155                 160

Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Val
                165                 170                 175

Arg Gln Asp Ser Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr
            180                 185                 190

Ala Ser Asp Pro Thr His Ile Ser Leu Asn Tyr Ala Leu Phe Thr Ser
        195                 200                 205
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Thr Ala Pro Val Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe
    210                 215                 220

Asp Gly Met Val Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe
225                 230                 235                 240

Gly Gln Ile Thr Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly
                245                 250                 255

Asn Glu Pro Tyr Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn
            260                 265                 270

Leu Leu Asn His Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr
        275                 280                 285

Ile Met Pro Thr Phe Phe Phe Glu Met Phe Asn Glu Asn Leu Lys Gln
    290                 295                 300

Pro Thr Val Glu Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro
305                 310                 315                 320

Val Tyr Pro Phe Trp
                325


<210> SEQ ID NO 3
<211> LENGTH: 1185
<212> TYPE: DNA
<213> ORGANISM: Gossypium hirsutum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (101)..(1078)

<400> SEQUENCE: 3

gcaccagtta ttgactttcc tttgtaaaaa aaaaaagtgc tgagatcaag aaatatagtg      60

aaatatgggt ccaagatttt ctgggttttt aatctaagca atg ctg ttt tta act       115
                                            Met Leu Phe Leu Thr
                                            1               5

caa ctc ctc tct cta aca gat ggc cgt gat att ggt gtt tgc tat ggt       163
Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys Tyr Gly
                10                  15                  20

ttg aac ggc aac aat ctt cca tct cca gga gat gtt att aat ctt ttc       211
Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Phe
            25                  30                  35

aaa act agt ggc ata aac aat atc agg ctc tac cag cct tac cct gaa       259
Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu
        40                  45                  50

gtg ctc gaa gca gca agg gga tcg gga ata tcc ctc tcg atg agt acg       307
Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Ser Thr
    55                  60                  65

aca aac gag gac ata caa agc ctc gca acg gat caa agt gca gcc gat       355
Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Ser Ala Ala Asp
70                  75                  80                  85

gca tgg gtt aac acc aac atc gtc cct tat aag gaa gat gtt caa ttc       403
Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Glu Asp Val Gln Phe
                90                  95                  100

agg ttc atc atc att ggg aat gaa gcc att cca gga cag tca agc tct       451
Arg Phe Ile Ile Ile Gly Asn Glu Ala Ile Pro Gly Gln Ser Ser Ser
            105                 110                 115

tac att cct ggt gcc atg aac aac ata atg aac tcg ctg gcc tca ttt       499
Tyr Ile Pro Gly Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser Phe
        120                 125                 130

ggg cta ggc acg acg aag gtt acg acc gtg gtc ccg atg aat gcc cta       547
Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu
    135                 140                 145

agt acc tcg tac cct cct tca gac ggc gct ttt gga agc gat ata aca       595
Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr
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150                 155                 160                 165

tcg atc atg act agt atc atg gcc att ctg gtt cga cag gat tcg ccc       643
Ser Ile Met Thr Ser Ile Met Ala Ile Leu Val Arg Gln Asp Ser Pro
                170                 175                 180

ctc ctg atc aat gtg tac cct tat ttt gcc tat gcc tca gac ccc act       691
Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr
            185                 190                 195

cat att tcc ctc aac tac gcc ttg ttc acc tcg acc gca ccg gtg gtg       739
His Ile Ser Leu Asn Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val
        200                 205                 210

gtc gac caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg gtc gat       787
Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp
    215                 220                 225

gct ttc aat gcc gcc cta gat aag atc ggc ttc ggc caa att act ctc       835
Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile Thr Leu
230                 235                 240                 245

att gta gcc gaa act gga tgg ccg acc gcc ggt aac gag cct tac acg       883
Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro Tyr Thr
                250                 255                 260

agt gtc gcg aac gct caa act tat aac aag aac ttg ttg aat cat gtg       931
Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn His Val
            265                 270                 275

acg cag aaa ggg act ccg aaa aga cct gaa tat ata atg ccg acg ttt       979
Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro Thr Phe
        280                 285                 290

ttc ttc gag atg ttc aac gag aac ttg aag caa ccc aca gtt gag cag      1027
Phe Phe Glu Met Phe Asn Glu Asn Leu Lys Gln Pro Thr Val Glu Gln
    295                 300                 305

aat ttc gga ttc ttc ttc ccc aat atg aac cct gtt tat cca ttt tgg      1075
Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro Phe Trp
310                 315                 320                 325

tga acttgaaatg ttattgttgg ctatttaaat cttttgccag agacgcttca           1128

tatagtttct gcatattttg aaagtggaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa       1185


<210> SEQ ID NO 4
<211> LENGTH: 325
<212> TYPE: PRT
<213> ORGANISM: Gossypium hirsutum

<400> SEQUENCE: 4

Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile
1               5                   10                  15

Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp
            20                  25                  30

Val Ile Asn Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr
        35                  40                  45

Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser
    50                  55                  60

Leu Ser Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp
65                  70                  75                  80

Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys
                85                  90                  95

Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu Ala Ile Pro
            100                 105                 110

Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn Ile Met Asn
        115                 120                 125

Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val
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    130                 135                 140

Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe
145                 150                 155                 160

Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Val
                165                 170                 175

Arg Gln Asp Ser Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr
            180                 185                 190

Ala Ser Asp Pro Thr His Ile Ser Leu Asn Tyr Ala Leu Phe Thr Ser
        195                 200                 205

Thr Ala Pro Val Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe
    210                 215                 220

Asp Gly Met Val Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe
225                 230                 235                 240

Gly Gln Ile Thr Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly
                245                 250                 255

Asn Glu Pro Tyr Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn
            260                 265                 270

Leu Leu Asn His Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr
        275                 280                 285

Ile Met Pro Thr Phe Phe Phe Glu Met Phe Asn Glu Asn Leu Lys Gln
    290                 295                 300

Pro Thr Val Glu Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro
305                 310                 315                 320

Val Tyr Pro Phe Trp
                325


<210> SEQ ID NO 5
<211> LENGTH: 1185
<212> TYPE: DNA
<213> ORGANISM: Gossypium barbadense
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (63)..(96)
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (209)..(711)

<400> SEQUENCE: 5

gctgagatca agaaatatag tgaaatatgg gtccaagatt ttctgggttt ttaatctaag      60

ca atg ctg ttt tta act caa ctc ctc tct cta aca g gtaaaacaaa          106
   Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr
   1               5                   10

cttctctaca gtgattttac agtaaatatg gctttgaaaa atatacaaca aaacatttat    166

cttcaatcca ttttaattac tgatctacta tatatgttgc ag at  ggc cgt gat       219
                                               Asp Gly Arg Asp
                                               15

att ggt gtt tgc tat ggt ttg aac ggc aac aat ctt cca tct cca gga      267
Ile Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly
                20                  25                  30

gat gtt att aat ctt ttc aaa act agt ggc ata aac aat atc agg ctc      315
Asp Val Ile Asn Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu
            35                  40                  45

tac cag cct tac cct gaa gtg ctc gaa gca gca agg gga tcg gga ata      363
Tyr Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile
        50                  55                  60

tcc ctc tcg atg agt acg aca aac gag gac ata caa agc ctc gca acg      411
Ser Leu Ser Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr
    65                  70                  75
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gat caa act cat caa agt gca gcc gat gca tgg gtt aac acc aac atc      459
Asp Gln Thr His Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile
80                  85                  90                  95

gtc cct tat aag gaa gat gtt caa ttc agg ttc atc atc att ggg aat      507
Val Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn
                100                 105                 110

gaa gcc att cca gga cag tca agc tct tac att cct ggt gcc atg aac      555
Glu Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn
            115                 120                 125

aac ata atg aac tcg ctc gcc tca ttt ggg cta ggc acg acg aag gtt      603
Asn Ile Met Asn Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val
        130                 135                 140

acg acc gtg gtc ccg atg aat gcc cta agt acc tcg tac cct cct tca      651
Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser
    145                 150                 155

gac ggc gct ttt gga agc gat ata aca tcg atc atg act agt atc atg      699
Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met
160                 165                 170                 175

gcc att ctg gtt tgacaggatt cgcccctcct gatcaatgtg taccottatt          751
Ala Ile Leu Val

ttgcctatgc ctcagacccc actcatattt ccctcaacta cgccttgttc acctcgaccg    811

caccggtggt ggtcgaccaa cgcttggaat actacaacct ctttgacggc atagtcgatg    871

ctttcaatgc cgccctagat aagatcggct tcggccaaat tactctcatt gtagccgaaa    931

ctggatggcc gaccgccggt aacgagcctt acacgagtgt cgcgaacgct caaacttata    991

acaagaactt gttgaatcat gtgacgcaga aagggactcc gaaaagacct gaatatataa   1051

tgccgacgtt tttcttcgag atgttcaacg agaacttgaa gcaacccaca gttgagcaga   1111

tgttcaacga gatgttcaac gagaacttga aatgttattg ttggctattt aaatcttttg   1171

ccagagacgc ttca                                                     1185


<210> SEQ ID NO 6
<211> LENGTH: 179
<212> TYPE: PRT
<213> ORGANISM: Gossypium barbadense

<400> SEQUENCE: 6

Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile
1               5                   10                  15

Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp
            20                  25                  30

Val Ile Asn Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr
        35                  40                  45

Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser
    50                  55                  60

Leu Ser Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp
65                  70                  75                  80

Gln Thr His Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val
                85                  90                  95

Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu
            100                 105                 110

Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn
        115                 120                 125

Ile Met Asn Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr
    130                 135                 140
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Thr Val Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp
145                 150                 155                 160

Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala
                165                 170                 175

Ile Leu Val


<210> SEQ ID NO 7
<211> LENGTH: 6877
<212> TYPE: DNA
<213> ORGANISM: Gossypium hirsutum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (3337)..(3406)
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (3501)..(4441)

<400> SEQUENCE: 7

ttcaaactta ctcgcttgca caaaaataat tttataaaag tatttaaaat ataaaatttt     60

tatgttgata atatttttat atacatttta tattttaaga aataataatt ttttaggaat    120

tagaaaaaaa atgtagaata atatcattga tattttaatt tttcaaaaaa ttaaaaataa    180

gttcacgtag tctaatttta tctattttaa tttttatact ttcaaattga gataaatatc    240

aaagaacttt tggttcaata tgcaatttga tacttaaatt ttaatttgat gtaattatta    300

catgaaactt ggcttgtggt ttatacgtat acatgaaatt tttattttga ttcaattgta    360

cgcatttaaa gaaatgaaaa tggttctaat tcaataatat tattagtgat ttgtgaaatt    420

taaaactttt atgcattaaa ccacacaaaa tcagagttta tgtatgatat tgcacattgg    480

actatagttc atgcatattt tttatatttt atccatgtca aattttgaaa tttcattctt    540

aacttatatg atagcagtta aatttgttaa gtcaaactct agtattagtt atatactata    600

cataacttgt agagtttagt ttaagttcac taatttgatt attttttatc tgtttatttt    660

ttcaatttca agatttaagt tttaagctta acttaaacaa tagtcattaa atttattaac    720

taaaatgtcc tggggttttt tgtaagtatt ataatatgtt tgccacgtga gattttggta    780

aaagtagagt ttaacttaac aaatttaatg gctactactt agtaaggatt agaatttcaa    840

aattaaaaaa aaaattatag aggctaaaga tgatcaaatt agaggtttaa attaagtcaa    900

attaaaatag ttctggatat taactattta aattaattaa tgtcattata aaattagagg    960

tctaaattat gtaaaattaa aatataaaaa ctaaatctcg aatgtgagta tagtataagg   1020

atcaaaagtg attttggtca ttttctttta tttacaaata ttcttagaga tgctctttta   1080

tatataatgg tttctaatgt gatatgcgcg gcatataatt ttatgtgttt aatttatttt   1140

attaattatt taaataatat atttttaatt actataatta atgtaaaata tttttttatta   1200

tttgaatcac tgcacaaaat taaaatatac taacttaatt aacaattcaa atataataat   1260

aatctaaatt ataattaaag catttttaca atattcagta tataatatag ttttacttta   1320

tataattaat acaaagaaat attattcaaa ataataacta atcaacataa ttactatata   1380

tcaattattt tgatatttcg aacataatgc taataaaaaa tttcctaatc attattaaat   1440

catttgtata aactataaag aaattgatat attgtaaatt aaacttttaa ctattcaatt   1500

ttttcttaat agtcaataaa ttaatcataa taattcataa ttaatatata attaacataa   1560

ccataacata gaatttttta ttttggccca ttaaaatttt taaaatttta aattagtaaa   1620

ggaaaaatta cactttgacc ccttaaaaat gataaaattt tattttaatc ctttaaaatt   1680

gacattttta ctattgtaaa aattacaatt taattttgcc cccctaaaaa atttttctag   1740
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cttcgccctt gtgtataaat atattaatta caatttttat atttgaatta tataaataat   1800

taaattttga tatttaaaac taaagtaatc tctttttttt ttactttttt ttaattgaaa   1860

cataatggtt taaatatcta tattacgtat gaagtaattt aatataaatt ttattttaat   1920

ttattattat ataaattcat ttagtaaaaa cttttaatag aatcaaaatt tttatttgta   1980

aattcgataa cttttcttat caagtaaatt tgttgaatta aatatttagt aaaattaata   2040

tttttattta taaatatgat aaatcttata aaaaataaaa aaatatttaa aatgaaaaac   2100

attgtacaaa ggctatataa gaagttcaaa agtttcttcg accctgtact ctaatagaga   2160

ttatagatag attatagaac tattcatagt ttctcttaac ctttaaataa gaattttagt   2220

gtactcaaac ttacatattt ttatattgat aataatgtca ataccagccg agttaagatt   2280

cactcgacat taatgttgaa aatttttaat aaaagaaaat gttgataagt taattagaac   2340

acaagcaagc acaaatttaa gtggtaagta aggtccttga ccctaatgga aaaattgtta   2400

tgttgattaa attataaatt aatttaaggt aaaattatat tttgacctaa aaaaatgaaa   2460

aaaatatatc tagtttcttc gaaaatgaaa agaaaaataat aaattgatac attataaaat   2520

ttatggcatt tctaaaaaaa ttctgaattt gatgaaatta taataaaaaa aaagtttaaa   2580

aacatataga tttcaagaat agtgggaaaa ttatatttga acaacactga agaaatccaa   2640

agcattagca gaaaatggat caccaaatca caaacaatca gtgaaaagta atgataatta   2700

attgaaagtg agcatttaaa tttgatagcc atatacttcc tgctgaattt ataggttctc   2760

attaatgcaa ttaaattata tttgtcactt tttgaatgaa ataaatgaca cagttcatct   2820

atttttttc tttcaatcgc ccatcaaaat accgaaaatg taactacatt aaaaaagatc   2880

gaaaaatatt catattttga tattttaata gattgtgtgt tcaaggcgta atgtactaaa   2940

aaattatgat ggtgttgtcg ctgtatgtcc ataaaattca atgtattcgc atgtatcaaa   3000

tgtaaatttt gacacaagtt attctaataa taatcaagtt atttttatac atgagataca   3060

tctcaaaatt atttttatat atccgaaaaa tcataacgta cgatcaaact agaaagagga   3120

agtgtcaaaa cctattcatt atatgcaaat atgatgggac acgataccct catgcattga   3180

tatctcatat tgtccaaaaa ctcagaatcc tttttgaaaa aaaaaaattc cagagagagt   3240

gtataaatcc agcagtgtgc acaagaaacg agcaccagtt attgacattc ctttgtaaaa   3300

aaaaaaagaa gctgagatca agaaatatag tgaaat atg ggt cca aca ttt tct     3354
                                        Met Gly Pro Thr Phe Ser
                                        1               5

ggg ttt tta atc tca gca atg gtg ttt tta act caa ctc ctc tct cta     3402
Gly Phe Leu Ile Ser Ala Met Val Phe Leu Thr Gln Leu Leu Ser Leu
            10                  15                  20

aca g gtaaaacaaa cttctctaca gtgattttac ggtaagtatg gctttgaaaa        3456
Thr

atatacaaca aaacatttat actgatctac catatatgtt gcag at  ggc cgt gat    3511
                                                 Asp Gly Arg Asp
                                                     25

att ggt gtt tgc tat ggt ttg aac ggc aac aat ctt cca tct cca gga     3559
Ile Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly
        30                  35                  40

gat gtt att aat ctt tac aaa act agt ggc ata aac aat atc agg ctc     3607
Asp Val Ile Asn Leu Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu
    45                  50                  55

tac cag cct tac cct gaa gtg ctc gaa gca gca agg gga tcg gga ata     3655
Tyr Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile
60                  65                  70                  75
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tcc ctc tcg atg ggt ccg aga aac gag gac ata caa agc ctc gca aaa     3703
Ser Leu Ser Met Gly Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys
                80                  85                  90

gat caa agt gca gcc gat gca tgg gtt aac acc aac atc gtc cct tat     3751
Asp Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr
            95                  100                 105

aag gac gat gtt cag ttc aag ttg atc act att ggg aat gaa gcc att     3799
Lys Asp Asp Val Gln Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile
        110                 115                 120

tca gga caa tca agc tct tac att cct gat gcc atg aac aac ata atg     3847
Ser Gly Gln Ser Ser Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met
    125                 130                 135

aac tcg ctc gcc tta ttt ggg tta ggc acg acg aag gtt acg acc gtg     3895
Asn Ser Leu Ala Leu Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val
140                 145                 150                 155

gtc ccg atg aat gcc cta agt acc tcg tac cct cct tca gac ggc gct     3943
Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala
                160                 165                 170

ttt gga agc gat ata aca tcg atc atg act agt atc atg gcc att ctg     3991
Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu
            175                 180                 185

gct gta cag gat tcg ccc ctc ctg atc aat gtg tac cct tat ttt gcc     4039
Ala Val Gln Asp Ser Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala
        190                 195                 200

tat gcc tca gac ccc act cat att tcc ctc gat tac gcc ttg ttc acc     4087
Tyr Ala Ser Asp Pro Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr
    205                 210                 215

tcg acc gca ccg gtg gtg gtc gac caa ggc ttg gaa tac tac aac ctc     4135
Ser Thr Ala Pro Val Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu
220                 225                 230                 235

ttt gac ggc atg gtc gat gct ttc aat gcc gcc cta gat aag atc ggc     4183
Phe Asp Gly Met Val Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly
                240                 245                 250

ttc ggc caa att act ctc att gta gcc gaa act gga tgg ccg acc gcc     4231
Phe Gly Gln Ile Thr Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala
            255                 260                 265

ggt aac gag cct tac acg agt gtc gcg aac gct caa act tat aac aag     4279
Gly Asn Glu Pro Tyr Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys
        270                 275                 280

aac ttg tta aat cat gtg acg cag aag ggg act ccg aaa aga cct gaa     4327
Asn Leu Leu Asn His Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu
    285                 290                 295

tat ata atg ccg acg ttt ttc ttc gag atg ttc aac gag gat ttg aag     4375
Tyr Ile Met Pro Thr Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys
300                 305                 310                 315

caa ccc aca gtt gag cag aat ttc gga ttc ttc ttc ccc aat atg aac     4423
Gln Pro Thr Val Glu Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn
                320                 325                 330

cct gtt tat cca ttt tgg tgaagttgaa atgttgttgg ctatttaaat            4471
Pro Val Tyr Pro Phe Trp
            335

cttttgccag agacgcttca tatagtttct gcatattttg aaagtggaaa atcaatctaa   4531

atattaataa gttttatgtg ttgtttttta attaaataaa attttaaata ttttaaaaat   4591

atctttattg gtaattaaat attaaataaa aagtttaata ttcaaatttt atcaattcaa   4651

aaataaaata aaaatatatt aaatttattt ttacgaataa attgattttc tattaataca   4711

gattttgaat aatttgatat aaattttaaa ttcaacaata gtaattttga tcacatcaaa   4771
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ggagaaaggg aaagatttaa ctttaattgg tgacctaata taacacgttg aaaacggagc   4831
tcccaggaag gcaaaatgac ttgtaatgac gaaagagatg tccaagtaga atctgcatta   4891
aagtgaaaaa agcataaaag gataagtaaa ctcatgatct gacataaatt gaagttctat   4951
aaaatgcaac tttcatctag aaacaaggta tgtcttaaat gatgttttat gaatttgtct   5011
taactgggtt ttatgcaatg aattcatgga tagcacctca ctaattatac gttgctggtt   5071
tatatgagag tggtgcagaa gttaattgtg ctttaaatac ttgcttagtg ttcaagaaat   5131
ttgaaaagta ttatatattt ataataaaaa taattcagat ccgactcaat ctagtaaaat   5191
tttacaaaca ttctaaaggg gatcttcttt tttctctact tattgatcag tgttatatac   5251
ttataataaa gacaacctga tttgagatcc ggcctaatat aataaaattc tacagacatc   5311
tcaagggaga gatcttcttc ttccctacat cttgaccttt ttgatcaaaa tttcctcccc   5371
tctatttcca cattggttga tcatatgaat caacagaaag gtaccaaaaa gtttttaaaa   5431
ataaacaaag gggttcttat gaaattcata tgatatattg ggtctaatta ttagaatcaa   5491
ttttaagttt aaacaaattt aaaattcaaa actcaattcc atttttgttt gaacggaaag   5551
ttactaattg ttaagaaaaa taattcatat tagcgtataa attggaaatt gaccaaaact   5611
aaaattattg tatagttaat ctatattaaa aggacatgta attaaaaacc attaaaacta   5671
ttatagaata aattaaatct tcattctata catacaaagt cattaataat taaaaaacta   5731
tattaagata taaactatat tcaaaaaata ttaaaaacaa taactaaata aaaaaaacaa   5791
ttgaaaatta cgaattaatg ttaaaatcaa gggacttaaa taaaaatatc ccaaaataca   5851
aaacattagc ttcctttccc atccacgtga ttgcaaagtt tacatggtgt ttcctagtgc   5911
ttgtgcgact ccaacctttt atttactttt ttcttttctt tatttgaaca attatttgat   5971
aatgattaga attttgggat tgttgctcat cgtacgtgca acacttaaaa tcactatgat   6031
ttttcataat ttatataacc tatatcgttt tggaaattaa tgttattatt tatattgttt   6091
taataaaaat accatctacc tcttttaatt tatgatccat ttcttatttg aaaattcaaa   6151
ttgacagttg tctaactaaa caccatcgca ctccaataaa attgtaattt tttctatcgt   6211
gaatagtaca ctcaaaagta tgttgttaac aaacaaatca attagccttt ttctacctct   6271
attcatcatc ttcttaatag cgtgtttatg tcacgtgttg agattttagt tccggtcacg   6331
tgtggcctta aacccgaatt tcttacgcat gagtctaagt tagcctctga tcctcgctat   6391
ggagatgctt ggcacagttt acctaggtaa gtaaacaagg aatagagcta ttagaaagca   6451
tcagagagtt aggagaatgt ggaagtgttt ctattactca aagctaactt ggatacaaat   6511
aaaagaggga gcctctcctt taggcaagcc tattttgatc tgacggttgc aattaatctc   6571
gaataggagg ggtcgaactt ctcactcagt ttcacattat ctcttggtgc ttagttggcc   6631
tccgccttga gacacattca aataacacct agtcttaaca cttttggctt cttattgtgc   6691
gtatccttca ttactcaaat gccacaaagc ctcattactt aagactctcg gtcgctccca   6751
ctaccttcga ctttagactc atctaagatc ttcccaatcg tagacaactt ggccttggtg   6811
gggaaatctt gcaccctacg gggccttaca taagaagcaa ttaaatggct ttctctcacc   6871
cacctt                                                              6877
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<210> SEQ ID NO 8
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Gossypium hirsutum

<400> SEQUENCE: 8

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Met Gly Pro Thr Phe Ser Gly Phe Leu Ile Ser Ala Met Val Phe Leu
1               5                   10                  15

Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys Tyr
            20                  25                  30

Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu
        35                  40                  45

Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro
    50                  55                  60

Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly
65                  70                  75                  80

Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala
                85                  90                  95

Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln
            100                 105                 110

Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser
        115                 120                 125

Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu
    130                 135                 140

Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
145                 150                 155                 160

Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
                165                 170                 175

Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser
            180                 185                 190

Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro
        195                 200                 205

Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val
    210                 215                 220

Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val
225                 230                 235                 240

Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile Thr
                245                 250                 255

Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro Tyr
            260                 265                 270

Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn His
        275                 280                 285

Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro Thr
    290                 295                 300

Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys Gln Pro Thr Val Glu
305                 310                 315                 320

Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro Phe
                325                 330                 335

Trp
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<210> SEQ ID NO 9
<211> LENGTH: 1250
<212> TYPE: DNA
<213> ORGANISM: Gossypium hirsutum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (66)..(1076)

<400> SEQUENCE: 9

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gcaccagtta ttgacattcc tttgtaaaaa aaaaaagaag ctgagatcaa gaaatatagt      60
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gaaat atg ggt cca aca ttt tct ggg ttt tta atc tca gca atg gtg ttt     110
      Met Gly Pro Thr Phe Ser Gly Phe Leu Ile Ser Ala Met Val Phe
      1               5                   10                  15

tta act caa ctc ctc tct cta aca gat ggc cgt gat att ggt gtt tgc     158
Leu Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys
                20                  25                  30

tat ggt ttg aac ggc aac aat ctt cca tct cca gga gat gtt att aat     206
Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn
            35                  40                  45

ctt tac aaa act agt ggc ata aac aat atc agg ctc tac cag cct tac     254
Leu Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr
        50                  55                  60

cct gaa gtg ctc gaa gca gca agg gga tcg gga ata tcc ctc tcg atg     302
Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met
    65                  70                  75

ggt ccg aga aac gag gac ata caa agc ctc gca aaa gat caa agt gca     350
Gly Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala
80                  85                  90                  95

gcc gat gca tgg gtt aac acc aac atc gtc cct tat aag gac gat gtt     398
Ala Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val
                100                 105                 110

cag ttc aag ttg atc act att ggg aat gaa gcc att tca gga caa tca     446
Gln Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser
            115                 120                 125

agc tct tac att cct gat gcc atg aac aac ata atg aac tcg ctc gcc     494
Ser Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala
        130                 135                 140

tta ttt ggg tta ggc acg acg aag gtt acg acc gtg gtc ccg atg aat     542
Leu Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn
    145                 150                 155

gcc cta agt acc tcg tac cct cct tca gac ggc gct ttt gga agc gat     590
Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp
160                 165                 170                 175

ata aca tcg atc atg act agt atc atg gcc att ctg gct gta cag gat     638
Ile Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp
                180                 185                 190

tcg ccc ctc ctg atc aat gtg tac cct tat ttt gcc tat gcc tca gac     686
Ser Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp
            195                 200                 205

ccc act cat att tcc ctc gat tac gcc ttg ttc acc tcg acc gca ccg     734
Pro Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro
        210                 215                 220

gtg gtg gtc gac caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg     782
Val Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met
    225                 230                 235

gtc gat gct ttc aat gcc gcc cta gat aag atc ggc ttc ggc caa att     830
Val Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile
240                 245                 250                 255

act ctc att gta gcc gaa act gga tgg ccg acc gcc ggt aac gag cct     878
Thr Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro
                260                 265                 270

tac acg agt gtc gcg aac gct caa act tat aac aag aac ttg tta aat     926
Tyr Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn
            275                 280                 285

cat gtg acg cag aag ggg act ccg aaa aga cct gaa tat ata atg ccg     974
His Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro
        290                 295                 300

acg ttt ttc ttc gag atg ttc aac gag gat ttg aag caa ccc aca gtt    1022
Thr Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys Gln Pro Thr Val
    305                 310                 315
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gag cag aat ttc gga ttc ttc ttc ccc aat atg aac cct gtt tat cca      1070
Glu Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro
320                 325                 330                 335

ttt tgg tgaagttgaa atgttgttgg ctatttaaat cttttgccag agacgcttca      1126
Phe Trp

tatagtttct gcatattttg aaagtggaaa atcaatctaa atattaataa gttttatgtg   1186

ttgtttttta attaaataaa attttaaata ttataaaaaa aaaaaaaaaa aaaaaaaaaa   1246

aaaa                                                                 1250


<210> SEQ ID NO 10
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Gossypium hirsutum

<400> SEQUENCE: 10

Met Gly Pro Thr Phe Ser Gly Phe Leu Ile Ser Ala Met Val Phe Leu
1               5                   10                  15

Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys Tyr
            20                  25                  30

Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu
        35                  40                  45

Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro
    50                  55                  60

Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly
65                  70                  75                  80

Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala
                85                  90                  95

Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln
            100                 105                 110

Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser
        115                 120                 125

Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu
    130                 135                 140

Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
145                 150                 155                 160

Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
                165                 170                 175

Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser
            180                 185                 190

Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro
        195                 200                 205

Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val
    210                 215                 220

Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val
225                 230                 235                 240

Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile Thr
                245                 250                 255

Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro Tyr
            260                 265                 270

Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn His
        275                 280                 285

Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro Thr
    290                 295                 300
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```
Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys Gln Pro Thr Val Glu
305                 310                 315                 320

Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro Phe
                325                 330                 335

Trp


<210> SEQ ID NO 11
<211> LENGTH: 1186
<212> TYPE: DNA
<213> ORGANISM: Gossypium barbadense
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (27)..(96)
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (191)..(1131)

<400> SEQUENCE: 11

gctgagatca agaaatatag tgaaat atg ggt cca aca ttt tct ggg ttt tta      53
                             Met Gly Pro Thr Phe Ser Gly Phe Leu
                             1               5

atc tca gca atg gtg ttt tta act caa ctc ctc tct cta aca g             96
Ile Ser Ala Met Val Phe Leu Thr Gln Leu Leu Ser Leu Thr
10                  15                  20

gtaaaacaaa cttctctaca gtgattttac ggtaagtatg gctttgaaaa atatacaaca    156

aaacatttat actgatctac catatatgtt gcag at  ggc cgt gat att ggt gtt    210
                                      Asp Gly Arg Asp Ile Gly Val
                                      25                  30

tgc tat ggt ttg aac ggc aac aat ctt cca tct cca gga gat gtt att      258
Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile
                35                  40                  45

aat ctt tac aaa act agt ggc ata aac aat atc agg ctc tac cag tct      306
Asn Leu Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Ser
            50                  55                  60

tac cct gaa gtg ctc gaa gca gca agg gga tcg gga ata tcc ctc tcg      354
Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser
        65                  70                  75

atg ggt ccg aga aac gag gac ata caa agc ctc gca aaa gat caa agt      402
Met Gly Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser
    80                  85                  90

gca gcc gat gca tgg gtt aac acc aac atc gtc cct tat aag gac gat      450
Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp
95                  100                 105                 110

gtt cag ttc aag ttg atc act att ggg aat gaa gcc att tca gga caa      498
Val Gln Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln
                115                 120                 125

tca agc tct tac att cct gat gcc atg aac aac ata atg aac tcg ctc      546
Ser Ser Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu
            130                 135                 140

gcc tta ttt ggg tta ggc acg acg aag gtt acg acc gtg gtc ccg atg      594
Ala Leu Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met
        145                 150                 155

aat gcc cta agt acc tcg tac cct cct tca gac ggc gct ttt gga agc      642
Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser
    160                 165                 170

gat ata aca tcg atc atg act agt atc atg gcc att ctg gct gta cag      690
Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln
175                 180                 185                 190

gat tcg ccc ctc ctg atc aat gtg tac cct tat ttt gcc tat gcc tca      738
Asp Ser Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser
                195                 200                 205
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```
gac ccc act cat att tcc ctc gat tac gcc ttg ttc acc tcg acc gca      786
Asp Pro Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala
            210                 215                 220

ccg gtg gtg gtc gac caa ggc ttg gaa tac tac aac ctc ttt gac ggc      834
Pro Val Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly
        225                 230                 235

atg gtc gat gct ttc aat gcc gcc cta gat aag atc ggc ttc ggc caa      882
Met Val Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln
    240                 245                 250

att act ctc att gta gcc gaa act gga tgg ccg acc gcc ggt aac gag      930
Ile Thr Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu
255                 260                 265                 270

cct tac acg agt gtc gcg aac gct caa act tat aac aag aac ttg tta      978
Pro Tyr Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu
                275                 280                 285

aat cat gtg acg cag aag ggg act ccg aaa aga cct gaa tat ata atg     1026
Asn His Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met
            290                 295                 300

ccg acg ttt ttc ttc gag atg ttc aac gag gat ttg aag caa ccc aca     1074
Pro Thr Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys Gln Pro Thr
        305                 310                 315

gtt gag cag aat ttc gga ttc ttc ttc ccc aat atg aac cct gtt tat     1122
Val Glu Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr
    320                 325                 330

cca ttt tgg tgaagttgaa atgttgttgg ctatttaaat cttttgccag             1171
Pro Phe Trp
335

agacgcttca tatag                                                    1186


<210> SEQ ID NO 12
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Gossypium barbadense

<400> SEQUENCE: 12

Met Gly Pro Thr Phe Ser Gly Phe Leu Ile Ser Ala Met Val Phe Leu
1               5                   10                  15

Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys Tyr
            20                  25                  30

Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu
        35                  40                  45

Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Ser Tyr Pro
    50                  55                  60

Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly
65                  70                  75                  80

Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala
                85                  90                  95

Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln
            100                 105                 110

Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser
        115                 120                 125

Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu
    130                 135                 140

Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
145                 150                 155                 160

Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
                165                 170                 175
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Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser
            180                 185                 190

Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro
        195                 200                 205

Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val
    210                 215                 220

Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val
225                 230                 235                 240

Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile Thr
                245                 250                 255

Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro Tyr
            260                 265                 270

Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn His
        275                 280                 285

Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro Thr
    290                 295                 300

Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys Gln Pro Thr Val Glu
305                 310                 315                 320

Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro Phe
                325                 330                 335

Trp


<210> SEQ ID NO 13
<211> LENGTH: 1211
<212> TYPE: DNA
<213> ORGANISM: Gossypium barbadense
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (29)..(1039)

<400> SEQUENCE: 13

ttgctgagat caagaaatat agtgaaat atg ggt cca aca ttt tct ggg ttt         52
                               Met Gly Pro Thr Phe Ser Gly Phe
                               1               5

tta atc tca gca atg gtg ttt tta act caa ctc ctc tct cta aca gat       100
Leu Ile Ser Ala Met Val Phe Leu Thr Gln Leu Leu Ser Leu Thr Asp
    10                  15                  20

ggc cgt gat att ggt gtt tgc tat ggt ttg aac ggc aac aat ctt cca       148
Gly Arg Asp Ile Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro
25                  30                  35                  40

tct cca gga gat gtt att aat ctt tac aaa act agt ggc ata aac aat       196
Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr Ser Gly Ile Asn Asn
                45                  50                  55

atc agg ctc tac cag tct tac cct gaa gtg ctc gaa gca gca agg gga       244
Ile Arg Leu Tyr Gln Ser Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly
            60                  65                  70

tcg gga ata tcc ctc tcg atg ggt ccg aga aac gag gac ata caa agc       292
Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn Glu Asp Ile Gln Ser
        75                  80                  85

ctc gca aaa gat caa agt gca gcc gat gca tgg gtt aac acc aac atc       340
Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile
    90                  95                  100

gtc cct tat aag gac gat gtt cag ttc aag ttg atc act att ggg aat       388
Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu Ile Thr Ile Gly Asn
105                 110                 115                 120

gaa gcc att tca gga caa tca agc tct tac att cct gat gcc atg aac       436
Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile Pro Asp Ala Met Asn
                125                 130                 135
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aac ata atg aac tcg ctc gcc tta ttt ggg tta ggc acg acg aag gtt      484
Asn Ile Met Asn Ser Leu Ala Leu Phe Gly Leu Gly Thr Thr Lys Val
            140                145                150

acg acc gtg gtc ccg atg aat gcc cta agt acc tcg tac cct cct tca      532
Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser
        155                160                165

gac ggc gct ttt gga agc gat ata aca tcg atc atg act agt atc atg      580
Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met
    170                175                180

gcc att ctg gct gta cag gat tcg ccc ctc ctg atc aat gtg tac cct      628
Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu Ile Asn Val Tyr Pro
185                190                195                200

tat ttt gcc tat gcc tca gac ccc act cat att tcc ctc gat tac gcc      676
Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile Ser Leu Asp Tyr Ala
                205                210                215

ttg ttc acc tcg acc gca ccg gtg gtg gtc gac caa ggc ttg gaa tac      724
Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp Gln Gly Leu Glu Tyr
            220                225                230

tac aac ctc ttt gac ggc atg gtc gat gct ttc aat gcc gcc cta gat      772
Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe Asn Ala Ala Leu Asp
        235                240                245

aag atc ggc ttc ggc caa att act ctc att gta gcc gaa act gga tgg      820
Lys Ile Gly Phe Gly Gln Ile Thr Leu Ile Val Ala Glu Thr Gly Trp
    250                255                260

ccg acc gcc ggt aac gag cct tac acg agt gtc gcg aac gct caa act      868
Pro Thr Ala Gly Asn Glu Pro Tyr Thr Ser Val Ala Asn Ala Gln Thr
265                270                275                280

tat aac aag aac ttg tta aat cat gtg acg cag aag ggg act ccg aaa      916
Tyr Asn Lys Asn Leu Leu Asn His Val Thr Gln Lys Gly Thr Pro Lys
                285                290                295

aga cct gaa tat ata atg ccg acg ttt ttc ttc gag atg ttc aac gag      964
Arg Pro Glu Tyr Ile Met Pro Thr Phe Phe Phe Glu Met Phe Asn Glu
            300                305                310

gat ttg aag caa ccc aca gtt gag cag aat ttc gga ttc ttc ttc ccc     1012
Asp Leu Lys Gln Pro Thr Val Glu Gln Asn Phe Gly Phe Phe Phe Pro
        315                320                325

aat atg aac cct gtt tat cca ttt tgg tgaagttgaa atgttgttgg           1059
Asn Met Asn Pro Val Tyr Pro Phe Trp
    330                335

ctatttaaat cttttgccag agacgctcca tatagtttct gcatattttg aaagtggaaa   1119

gtcaatctaa atattaataa gttttgtgtt gttttttaat taaataaaat tttaaatatt   1179

ttggaaaaaa aaaaaaaaaa aaaaaaaaaa aa                                  1211


<210> SEQ ID NO 14
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Gossypium barbadense

<400> SEQUENCE: 14

Met Gly Pro Thr Phe Ser Gly Phe Leu Ile Ser Ala Met Val Phe Leu
1               5                   10                  15

Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys Tyr
            20                  25                  30

Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu
        35                  40                  45

Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Ser Tyr Pro
    50                  55                  60
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Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly
65                  70                  75                  80

Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala
                85                  90                  95

Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln
            100                 105                 110

Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser
        115                 120                 125

Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu
    130                 135                 140

Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
145                 150                 155                 160

Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
                165                 170                 175

Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser
            180                 185                 190

Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro
        195                 200                 205

Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val
    210                 215                 220

Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val
225                 230                 235                 240

Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile Thr
                245                 250                 255

Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro Tyr
            260                 265                 270

Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn His
        275                 280                 285

Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro Thr
    290                 295                 300

Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys Gln Pro Thr Val Glu
305                 310                 315                 320

Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro Phe
                325                 330                 335

Trp


<210> SEQ ID NO 15
<211> LENGTH: 656
<212> TYPE: DNA
<213> ORGANISM: Gossypium tomentosum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2)..(655)

<400> SEQUENCE: 15

c ggc aac aat ctt cca tct cca gga gat gtt att gat ctt ttc aaa act      49
  Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asp Leu Phe Lys Thr
  1               5                   10                  15

agt ggc ata aac aat atc agg ctc tac cag cct tac cct gaa gtg ctc       97
Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

gaa gca gca agg gga tcg gga ata tcc ctc tcg atg agt acg aca aac      145
Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Ser Thr Thr Asn
        35                  40                  45

gag gac ata caa agc ctc gca acg gat caa agt gca gcc gat gca tgg      193
Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60
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gtt aac acc aac atc gtc cct tat aag gaa gat gtt caa ttc agg ttc      241
Val Asn Thr Asn Ile Val Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe
65                  70                  75                  80

atc atc att ggg aat gaa gcc att cca gga cag tca agc tct tac att      289
Ile Ile Ile Gly Asn Glu Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

cct ggt gcc atg aac aac ata atg aac tcg ctg gcc tca ttt ggg cta      337
Pro Gly Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser Phe Gly Leu
            100                 105                 110

ggc acg acg aag gtt acg acc gtg gtc ccg atg aat gcc cta agt acc      385
Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

tcg tac cct cct tca gac ggc gct ttt gga agc gat ata aca tcg atc      433
Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

atg act agt atc atg gcc att ctg gtt cga cag gat tcg ccc ctc ctg      481
Met Thr Ser Ile Met Ala Ile Leu Val Arg Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

atc aat gtg tac cct tat ttt gcc tat gcc tca gac ccc act cat att      529
Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

tcc ctc aac tac gcc ttg ttc acc tcg gcc gca ccg gtg gtg gtc gac      577
Ser Leu Asn Tyr Ala Leu Phe Thr Ser Ala Ala Pro Val Val Val Asp
            180                 185                 190

caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg gtc gat gct ttc      625
Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

aat gcc gcc cta gat aag atc ggc ttc ggc c                            656
Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


<210> SEQ ID NO 16
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Gossypium tomentosum

<400> SEQUENCE: 16

Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asp Leu Phe Lys Thr
1               5                   10                  15

Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Ser Thr Thr Asn
        35                  40                  45

Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

Val Asn Thr Asn Ile Val Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe
65                  70                  75                  80

Ile Ile Ile Gly Asn Glu Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

Pro Gly Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser Phe Gly Leu
            100                 105                 110

Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

Met Thr Ser Ile Met Ala Ile Leu Val Arg Gln Asp Ser Pro Leu Leu
145                 150                 155                 160
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Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

Ser Leu Asn Tyr Ala Leu Phe Thr Ser Ala Ala Pro Val Val Val Asp
            180                 185                 190

Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


<210> SEQ ID NO 17
<211> LENGTH: 665
<212> TYPE: DNA
<213> ORGANISM: Gossypium darwinii
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2)..(472)

<400> SEQUENCE: 17

c ggc aac aat ctt cca tct cca gga gat gtt att aat ctt ttc aaa act      49
  Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Phe Lys Thr
  1               5                   10                  15

agt ggc ata aac aat atc agg ctc tac cag cct tac cct gaa gtg ctc       97
Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

gaa gca gca agg gga tcg gga ata tcc ctc tcg atg agt acg aca aac      145
Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Ser Thr Thr Asn
        35                  40                  45

gag gac ata caa agc ctc gca acg gat caa act cat caa agt gca gcc      193
Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Thr His Gln Ser Ala Ala
    50                  55                  60

gat gca tgg gtt aac acc aac atc gtc cct tat aag gaa gat gtt caa      241
Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Glu Asp Val Gln
65                  70                  75                  80

ttc agg ttc atc atc att ggg aat gaa gcc att cca gga cag tca agc      289
Phe Arg Phe Ile Ile Ile Gly Asn Glu Ala Ile Pro Gly Gln Ser Ser
                85                  90                  95

tct tac att cct ggt gcc atg aac aac ata atg aac tcg ctc gcc tca      337
Ser Tyr Ile Pro Gly Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser
            100                 105                 110

ttt ggg cta ggc acg acg aag gtt acg acc gtg gtc ccg atg aat gcc      385
Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
        115                 120                 125

cta agt acc tcg tac cct cct tca gac ggc gct ttt gga agc gat ata      433
Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
    130                 135                 140

aca tcg atc atg act agt atc atg gcc att ctg gtt tga caggattcgc      482
Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Val
145                 150                 155

ccctcctgat caatgtgtac ccttattttg cctatgcctc agaccccact catatttccc    542

tcaactacgc cttgttcacc tcgaccgcac cggtggtggt cgaccaaggc ttggaatact    602

acaacctctt tgacggcata gtcgatgctt tcaatgccgc cctagataag atcggcttcg    662

gcc                                                                  665


<210> SEQ ID NO 18
<211> LENGTH: 156
<212> TYPE: PRT
<213> ORGANISM: Gossypium darwinii

<400> SEQUENCE: 18
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Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Phe Lys Thr
1               5                   10                  15

Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Ser Thr Thr Asn
        35                  40                  45

Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Thr His Gln Ser Ala Ala
    50                  55                  60

Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Glu Asp Val Gln
65                  70                  75                  80

Phe Arg Phe Ile Ile Ile Gly Asn Glu Ala Ile Pro Gly Gln Ser Ser
                85                  90                  95

Ser Tyr Ile Pro Gly Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser
            100                 105                 110

Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
        115                 120                 125

Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
    130                 135                 140

Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Val
145                 150                 155


<210> SEQ ID NO 19
<211> LENGTH: 656
<212> TYPE: DNA
<213> ORGANISM: Gossypium mustelinum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2)..(655)

<400> SEQUENCE: 19

c ggc aac aat ctt cca tct cca gga gat gtt att aat ctt tac aaa act      49
  Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
  1               5                   10                  15

agt ggc ata aac aat atc agg ctc tac cag cct tac cct gaa gtg ctc        97
Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

gaa gca gca agg gga tcg gga ata tcc ctc tcg atg agt acg aca aac       145
Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Ser Thr Thr Asn
        35                  40                  45

gag gac ata caa agc ctc gca acg gat caa agt gca gcc gat gca tgg       193
Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

gtt aac acc aac atc gtc cct tat aag gaa gat gtt caa ttc agg ttc       241
Val Asn Thr Asn Ile Val Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe
65                  70                  75                  80

atc atc att ggg aat gaa gcc att cca gga cag tca agc tct tac att       289
Ile Ile Ile Gly Asn Glu Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

cct ggt gcc atg aac aac ata atg aac tcg ctc gcc tca ttt ggg cta       337
Pro Gly Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser Phe Gly Leu
            100                 105                 110

ggc acg acg aag gtt acg acc gtg gtc ccg atg aat gcc cta agt acc       385
Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

tcg tac cct cct tca gac ggc gct ttt gga agc gat ata aca tcg atc       433
Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

atg act agt atc atg gcc att ctg gtt cga cag gat tcg ccc ctc ctg       481
Met Thr Ser Ile Met Ala Ile Leu Val Arg Gln Asp Ser Pro Leu Leu
```

```
145                 150                 155                 160

atc aat gtg tac cct tat ttt gcc tat gcc tca gac ccc act cat att      529
Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

tcc ctc aac tac gcc ttg ttc acc tcg acc gca ccg gtg gtg gtc gac      577
Ser Leu Asn Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg gtc gat gct ttc      625
Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

aat gcc gcc cta gat aag atc ggc ttc ggc c                            656
Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


<210> SEQ ID NO 20
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Gossypium mustelinum

<400> SEQUENCE: 20

Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
1               5                   10                  15

Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Ser Thr Thr Asn
        35                  40                  45

Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

Val Asn Thr Asn Ile Val Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe
65                  70                  75                  80

Ile Ile Ile Gly Asn Glu Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

Pro Gly Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser Phe Gly Leu
            100                 105                 110

Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

Met Thr Ser Ile Met Ala Ile Leu Val Arg Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

Ser Leu Asn Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


<210> SEQ ID NO 21
<211> LENGTH: 1206
<212> TYPE: DNA
<213> ORGANISM: Gossypium arboreum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (27)..(96)
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (209)..(372)
```

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<400> SEQUENCE: 21

gctgagatca agaaatatag tgaaat atg ggt cca aga ttt tct ggg ttt tta      53
                             Met Gly Pro Arg Phe Ser Gly Phe Leu
                             1               5

atc tca gca atg ctg ttt tta act caa ctc ctc tct cta aca g             96
Ile Ser Ala Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr
10                  15                  20

gtaaaacaaa cttctctaca gtgattttag agtaaatatg gctttgaaaa atatacaaca    156

aaacatttat cttcaatcca ttttaattac tgatctacta tatatgttgc ag at  ggc    213
                                                          Asp Gly
                                                          25

cgt gat att ggt gtt tgc tat ggt ttg aac ggc aac aat ctt cca tct      261
Arg Asp Ile Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser
            30                  35                  40

cca gga gat gtt att aat ctt tac aaa act agt ggc ata aac aat atc      309
Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr Ser Gly Ile Asn Asn Ile
        45                  50                  55

agg ctc tac cag cct tac ctg aag tgc tcg aag gag caa ggg gat cgg      357
Arg Leu Tyr Gln Pro Tyr Leu Lys Cys Ser Lys Glu Gln Gly Asp Arg
    60                  65                  70

gaa tat ccc tct cga tgagtacgac aaacgaggac atacaaagcc tcgcaacgga      412
Glu Tyr Pro Ser Arg
75

tcaaagtgca gccgatgcat gggttaacac caacatcgtc ccttataagg acgatgttca    472

attcaggttc atcatcattg ggaatgaagc cattccagga cagtcaagct cttacattcc    532

tggtgccatg aacaacataa tgaactcgct cgcctcattt gggctaggca cgacgaaggt    592

tacgaccgtg gtcccgatga atgccctaag tacctcgtac cctccttcag acggcgcttt    652

tggaagcgat ataacatcga tcatgactag tatcatggcc attctggttc gacaggattc    712

gcccctcctg atcaatgtgt acccttattt tgcctatgcc tcagacccca ctcatatttc    772

cctcaactac gccttgttca cctcgaccgc accggtggtg gtcgaccaag gcttggaata    832

ctacaacctc tttgacggca tggtcgatgc tttcaatgcc gccctagata agatcggctt    892

cggccaaatt actctcattg tagccgaaac tggatggccg accgccggta acgagcctta    952

cacgagtgtc gcgaacgctc aaacttataa caagaacttg ttgaatcatg tgacgcagaa   1012

agggactccg aaaagacctg aatatataat gccgacgttt ttcttcgaga tgttcaacga   1072

gaacttgaag caacccacag ttgagcagaa tttcggattc ttcttcccca atatgaaccc   1132

tgtttatcca ttttggtgaa cttgaaatgt tattgttggc tatttaaatc ttttgccaga   1192

gacgcttcat atag                                                     1206


<210> SEQ ID NO 22
<211> LENGTH: 78
<212> TYPE: PRT
<213> ORGANISM: Gossypium arboreum

<400> SEQUENCE: 22

Met Gly Pro Arg Phe Ser Gly Phe Leu Ile Ser Ala Met Leu Phe Leu
1               5                   10                  15

Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys Tyr
            20                  25                  30

Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu
        35                  40                  45

Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Leu
```

```
    50                  55                  60

Lys Cys Ser Lys Glu Gln Gly Asp Arg Glu Tyr Pro Ser Arg
65                  70                  75
```

<210> SEQ ID NO 23
<211> LENGTH: 1207
<212> TYPE: DNA
<213> ORGANISM: Gossypium herbaceum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (27)..(96)
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (209)..(1149)

<400> SEQUENCE: 23

```
gctgagatca agaaatatag tgaaat atg ggt cca aga ttt tct ggg ttt tta      53
                             Met Gly Pro Arg Phe Ser Gly Phe Leu
                             1               5

atc tca gca atg ctg ttt tta act caa ctc ctc tct cta aca g             96
Ile Ser Ala Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr
10                  15                  20

gtaaaacaaa cttctctaca gtgattttac agtaaatatg gctttgaaaa atatacaaca    156

aaacatttat cttcaatcca ttttaattac tgatctacta tatatgttgc ag at  ggc    213
                                                          Asp Gly
                                                          25

cgt gat att ggt gtt tgc tat ggt ttg aac ggc aac aat ctt cca tct      261
Arg Asp Ile Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser
                30                  35                  40

cca gga gat gct att aat ctt tac aaa act agt ggc ata aac aat atc      309
Pro Gly Asp Ala Ile Asn Leu Tyr Lys Thr Ser Gly Ile Asn Asn Ile
            45                  50                  55

agg ctc tac cag cct tac cct gaa gtg ctc gaa gca gca agg gga tcg      357
Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser
        60                  65                  70

gga ata tcc ctc tcg atg agt acg aca aac gag gac ata caa agc ctc      405
Gly Ile Ser Leu Ser Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu
    75                  80                  85

gca acg gat caa agt gca gcc gat gca tgg gtt aac acc aac atc gtc      453
Ala Thr Asp Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val
90                  95                  100                 105

cct tat aag gac gat gtt caa ttc agg ttc atc atc att ggg aat gaa      501
Pro Tyr Lys Asp Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu
                110                 115                 120

gcc att cca gga cag tca agc tct tac att cct ggt gcc atg aac aac      549
Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn
            125                 130                 135

ata atg aac tcg ctc gcc tca ttt ggg cta ggc acg acg aag gtt acg      597
Ile Met Asn Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr
        140                 145                 150

acc gtg gtc ccg atg aat gcc cta agt acc tcg tac cct cct tca gac      645
Thr Val Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp
    155                 160                 165

ggc gct ttt gga agc gat ata aca tcg atc atg act agt atc atg gcc      693
Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala
170                 175                 180                 185

att ctg gtt cga cag gat tcg ccc ctc ctg atc aat gtg tac cct tat      741
Ile Leu Val Arg Gln Asp Ser Pro Leu Leu Ile Asn Val Tyr Pro Tyr
                190                 195                 200

ttt gcc tat gcc tca gac ccc act cat att tcc ctc aac tac gcc ttg      789
Phe Ala Tyr Ala Ser Asp Pro Thr His Ile Ser Leu Asn Tyr Ala Leu
```

```
            205                 210                 215

ttc acc tcg acc gca ccg gtg gtg gtc gac caa ggc ttg gaa tac tac      837
Phe Thr Ser Thr Ala Pro Val Val Val Asp Gln Gly Leu Glu Tyr Tyr
        220                 225                 230

aac ctc ttt gac ggc atg gtc gat gct ttc aat gcc gcc cta gat aag      885
Asn Leu Phe Asp Gly Met Val Asp Ala Phe Asn Ala Ala Leu Asp Lys
    235                 240                 245

atc ggc ttc ggc caa att act ctc att gta gcc gaa act gga tgg ccg      933
Ile Gly Phe Gly Gln Ile Thr Leu Ile Val Ala Glu Thr Gly Trp Pro
250                 255                 260                 265

acc gcc ggt aac gag cct tac acg agt gtc gcg aac gct caa act tat      981
Thr Ala Gly Asn Glu Pro Tyr Thr Ser Val Ala Asn Ala Gln Thr Tyr
                270                 275                 280

aac aag aac ttg ttg aat cat gtg acg cag aaa ggg act ccg aaa aga     1029
Asn Lys Asn Leu Leu Asn His Val Thr Gln Lys Gly Thr Pro Lys Arg
            285                 290                 295

cct gaa tat ata atg ccg acg ttt ttc ttc gag atg ttc aac gag aac     1077
Pro Glu Tyr Ile Met Pro Thr Phe Phe Phe Glu Met Phe Asn Glu Asn
        300                 305                 310

ttg aag caa ccc aca gtt gag cag aat ttc gga ttc ttc ttc ccc aat     1125
Leu Lys Gln Pro Thr Val Glu Gln Asn Phe Gly Phe Phe Phe Pro Asn
    315                 320                 325

atg aac cct gtt tat cca ttt tgg tgagcttgaa atgttattgt tggctattta    1179
Met Asn Pro Val Tyr Pro Phe Trp
330                 335

aatcttttgc cagagacgct tcatatag                                      1207


<210> SEQ ID NO 24
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Gossypium herbaceum

<400> SEQUENCE: 24

Met Gly Pro Arg Phe Ser Gly Phe Leu Ile Ser Ala Met Leu Phe Leu
1               5                   10                  15

Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys Tyr
            20                  25                  30

Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Ala Ile Asn Leu
        35                  40                  45

Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro
    50                  55                  60

Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Ser
65                  70                  75                  80

Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Ser Ala Ala
                85                  90                  95

Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln
            100                 105                 110

Phe Arg Phe Ile Ile Ile Gly Asn Glu Ala Ile Pro Gly Gln Ser Ser
        115                 120                 125

Ser Tyr Ile Pro Gly Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser
    130                 135                 140

Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
145                 150                 155                 160

Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
                165                 170                 175

Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Val Arg Gln Asp Ser
            180                 185                 190
```

```
Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro
        195                 200                 205

Thr His Ile Ser Leu Asn Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val
    210                 215                 220

Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val
225                 230                 235                 240

Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile Thr
                245                 250                 255

Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro Tyr
            260                 265                 270

Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn His
        275                 280                 285

Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro Thr
    290                 295                 300

Phe Phe Phe Glu Met Phe Asn Glu Asn Leu Lys Gln Pro Thr Val Glu
305                 310                 315                 320

Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro Phe
                325                 330                 335

Trp


<210> SEQ ID NO 25
<211> LENGTH: 656
<212> TYPE: DNA
<213> ORGANISM: Gossypium tomentosum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2)..(655)

<400> SEQUENCE: 25

c ggc aac aat ctt cca tct cca gga gat gtt att aat ctt tac aaa act      49
  Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
  1               5                   10                  15

agt ggc ata aac aat atc agg ctc tac cag cct tac cct gaa gtg ctc       97
Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

gaa gca gca agg gga tcg gga ata tcc ctc tcg atg ggt ccg aga aac      145
Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn
        35                  40                  45

gag gac ata caa agc ctc gca aaa gat caa agt gca gcc gat gca tgg      193
Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

gtt aac acc aac atc gtc cct tat aag gac gat gtt cag ttc aag ttg      241
Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu
65                  70                  75                  80

atc act att ggg aat gaa gcc att tca gga caa tca agc tct tac att      289
Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

cct gat gcc atg aac aac ata atg aac tcg ctc gcc tta ttt ggg tta      337
Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu Phe Gly Leu
            100                 105                 110

ggc acg acg aag gtt acg acc gtg gtc ccg atg aat gcc cta agt acc      385
Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

tcg tac cct cct tca gac ggc gct ttt gga agc gat ata aca tcg atc      433
Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

atg act agt atc atg gcc att ctg gct gta cag gat tcg ccc ctc ctg      481
Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu
```

```
145                 150                 155                 160

atc aat gtg tac cct tat ttt gcc tat gcc tca gac ccc act cat att      529
Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

tcc ctc gat tac gcc ttg ttc acc tcg acc gca ccg gtg gtg gtc gac      577
Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg gtc gat gct ttc      625
Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

aat gcc gcc cta gat aag atc ggc ttc ggc c                            656
Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


&lt;210&gt; SEQ ID NO 26
&lt;211&gt; LENGTH: 218
&lt;212&gt; TYPE: PRT
&lt;213&gt; ORGANISM: Gossypium tomentosum

&lt;400&gt; SEQUENCE: 26

Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
1               5                   10                  15

Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn
        35                  40                  45

Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu
65                  70                  75                  80

Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu Phe Gly Leu
            100                 105                 110

Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


&lt;210&gt; SEQ ID NO 27
&lt;211&gt; LENGTH: 656
&lt;212&gt; TYPE: DNA
&lt;213&gt; ORGANISM: Gossypium darwinii
&lt;220&gt; FEATURE:
&lt;221&gt; NAME/KEY: CDS
&lt;222&gt; LOCATION: (2)..(655)

&lt;400&gt; SEQUENCE: 27
```

```
c ggc aac aat ctt cca tct cca gga gat gtt att aat ctt tac aaa act      49
  Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
  1               5                   10                  15

agt ggc ata aac aat atc agg ctc tac cag tct tac cct gaa gtg ctc       97
Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Ser Tyr Pro Glu Val Leu
            20                  25                  30

gaa gca gca agg gga tcg gga ata tcc ctc tcg atg ggt ccg aga aac      145
Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn
        35                  40                  45

gag gac ata caa agc ctc gca aaa gat caa agt gca gcc gat gca tgg      193
Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

gtt aac acc aac atc gtc cct tat aag gac gat gtt cag ttc aag ttg      241
Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu
65                  70                  75                  80

atc act att ggg aat gaa gcc att tca gga caa tca agc tct tac att      289
Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

cct gat gcc atg aac aac ata atg aac tcg ctc gcc tta ttt ggg tta      337
Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu Phe Gly Leu
            100                 105                 110

ggc acg acg aag gtt acg acc gtg gtc ccg atg aat gcc cta agt acc      385
Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

tcg tac cct cct tca gac ggc gct ttt gga agc gat ata aca tcg atc      433
Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

atg act agt atc atg gcc att ctg gct gta cag gat tcg ccc ctc ctg      481
Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

atc aat gtg tac cct tat ttt gcc tat gcc tca gac ccc act cat att      529
Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

tcc ctc gat tac gcc ttg ttc acc tcg acc gca ccg gtg gtg gtc gac      577
Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg gtc gat gct ttc      625
Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

aat gcc gcc cta gat aag atc ggc ttc ggc c                            656
Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215
```

<210> SEQ ID NO 28
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Gossypium darwinii

<400> SEQUENCE: 28

```
Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
1               5                   10                  15

Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Ser Tyr Pro Glu Val Leu
            20                  25                  30

Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn
        35                  40                  45

Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu
65                  70                  75                  80
```

```
Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu Phe Gly Leu
            100                 105                 110

Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


<210> SEQ ID NO 29
<211> LENGTH: 656
<212> TYPE: DNA
<213> ORGANISM: Gossypium mustelinum
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2)..(655)

<400> SEQUENCE: 29

c ggc aac aat ctt cca tct cca gga gat gtt att aat ctt tac aaa act      49
  Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
  1               5                   10                  15

agt ggc ata aac aat atc agg ctc tac cag cct tac cct gaa gtg ctc       97
Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

gaa gca gca agg gga tcg gga ata tcc ctc tcg atg ggt ccg aga aac      145
Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn
        35                  40                  45

gag gac ata caa agc ctc gca aaa gat caa agt gca gcc gat gca tgg      193
Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

gtt aac acc aac atc gtc cct tat aag gac gat gtt cag ttc aag ttg      241
Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu
65                  70                  75                  80

atc act att ggg aat gaa gcc att tca gga caa tca agc tct tac att      289
Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

cct gat gcc atg aac aac ata atg aac tcg ctc gcc tta ttt ggg tta      337
Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu Phe Gly Leu
            100                 105                 110

ggc acg acg aag gtt acg acc gtg gtc ccg atg aat gcc cta aat acc      385
Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Asn Thr
        115                 120                 125

tcg tac cct cct tca gac ggc gct ttt gga agc gat ata aca tcg atc      433
Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

atg act agt atc atg gcc att ctg gct gta cag gat tcg ccc ctc ctg      481
Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

atc aat gtg tac cct tat ttt gcc tat gcc tca gac ccc act cat att      529
```

```
Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

tcc ctc gat tac gcc ttg ttc acc tcg acc gca ccg gtg gtg gtc gac       577
Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg gtc gat gct ttc       625
Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

aat gcc gct cta gat aag atc ggc ttc ggc c                             656
Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


<210> SEQ ID NO 30
<211> LENGTH: 218
<212> TYPE: PRT
<213> ORGANISM: Gossypium mustelinum

<400> SEQUENCE: 30

Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
1               5                   10                  15

Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn
        35                  40                  45

Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu
65                  70                  75                  80

Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu Phe Gly Leu
            100                 105                 110

Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Asn Thr
        115                 120                 125

Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


<210> SEQ ID NO 31
<211> LENGTH: 656
<212> TYPE: DNA
<213> ORGANISM: Gossypium raimondii
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (2)..(655)

<400> SEQUENCE: 31

c ggc aac aat ctt cca tct cca gga gat gtt att aat ctt tac aaa act      49
  Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
  1               5                   10                  15
```

```
agt ggc ata aac aat atc agg ctc tac cag cct tac cct gaa gtg ctc       97
Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

gaa gca gca agg gga tcg gga ata tcc ctc tcg atg ggt ccg aga aac      145
Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn
        35                  40                  45

gag gac ata caa agc ctc gca aaa gat caa agt gca gcc gat gca tgg      193
Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

gtt aac acc aac atc gtc cct tat aag gac gat gtt cag ttc aaa ttg      241
Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu
65                  70                  75                  80

atc act att ggg aat gaa gcc att tca gga caa tca agc tct tac att      289
Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95

cct gat gcc atg aac aac ata atg aac tcg ctc gcc tca ttt ggg tta      337
Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser Phe Gly Leu
            100                 105                 110

ggc aca acg aag gtt acg acc gtg gtc ccg atg aat gcc cta agt acc      385
Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

tcg tac cct cct tca gac ggc gct ttt gga agc gat ata aca tcg atc      433
Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

atg act agt atc atg gcc att ctg gct gta cag gat tcg ccc ctc ctg      481
Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

atc aat gtg tac cct tat ttt gcc tat gcc tca gac ccc act cat att      529
Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

tcc ctc gat tac gcc ttg ttc acc tcg acc gca ccg gtg gtg gtc gac      577
Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg gtc gat gct ttc      625
Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

aat gcc gcc cta gat aag atc ggc ttc ggc c                            656
Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


&lt;210&gt; SEQ ID NO 32
&lt;211&gt; LENGTH: 218
&lt;212&gt; TYPE: PRT
&lt;213&gt; ORGANISM: Gossypium raimondii

&lt;400&gt; SEQUENCE: 32

Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu Tyr Lys Thr
1               5                   10                  15

Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr Pro Glu Val Leu
            20                  25                  30

Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly Pro Arg Asn
        35                  40                  45

Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala Asp Ala Trp
    50                  55                  60

Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln Phe Lys Leu
65                  70                  75                  80

Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser Ser Tyr Ile
                85                  90                  95
```

```
Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Ser Phe Gly Leu
            100                 105                 110

Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala Leu Ser Thr
        115                 120                 125

Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile
    130                 135                 140

Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser Pro Leu Leu
145                 150                 155                 160

Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro Thr His Ile
                165                 170                 175

Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val Val Val Asp
            180                 185                 190

Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val Asp Ala Phe
        195                 200                 205

Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly
    210                 215


<210> SEQ ID NO 33
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer SE077

<400> SEQUENCE: 33

gctgagatca agaaatatag tg                                            22


<210> SEQ ID NO 34
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer SE078

<400> SEQUENCE: 34

ctatatgaag cgtctctggc                                               20


<210> SEQ ID NO 35
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer SE002

<400> SEQUENCE: 35

ggccgaagcc gatcttatct agg                                           23


<210> SEQ ID NO 36
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer SE003

<400> SEQUENCE: 36

cggcaacaat cttccatctc cag                                           23


<210> SEQ ID NO 37
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer p1.3GlucaAf
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<400> SEQUENCE: 37

tatccctctc gatgagtacg ac 22

<210> SEQ ID NO 38
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: p1.3GlucaAr

<400> SEQUENCE: 38

cccaatgatg atgaacctga attg 24

<210> SEQ ID NO 39
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: probe TM249-GCM1

<400> SEQUENCE: 39

aactcgctcg cctca 15

<210> SEQ ID NO 40
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: probe TM249-GCV1

<400> SEQUENCE: 40

aactcgctgg cctca 15

<210> SEQ ID NO 41
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer TM249-GCF

<400> SEQUENCE: 41

cctggtgcca tgaacaacat aatg 24

<210> SEQ ID NO 42
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: primer TM249-GCR

<400> SEQUENCE: 42

cgtcgtgcct agcccaaa 18

<210> SEQ ID NO 43
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: AFLP primer P5

<400> SEQUENCE: 43

gactgcgtac atgcagaa 18

<210> SEQ ID NO 44

<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: AFLP primer M50

<400> SEQUENCE: 44

gatgagtcct gagtaacat 19

<210> SEQ ID NO 45
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: forward SSR primer NAU861

<400> SEQUENCE: 45

ccaaaacttg tcccattagc 20

<210> SEQ ID NO 46
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: reverse SSR primer NAU861

<400> SEQUENCE: 46

ttcatctgtt gccagatcc 19

<210> SEQ ID NO 47
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: forward SSR primer CIR401

<400> SEQUENCE: 47

tggcgactcc ctttt 15

<210> SEQ ID NO 48
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: reverse SSR primer CIR401

<400> SEQUENCE: 48

aaaagatgtt acacacacac ac 22

<210> SEQ ID NO 49
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: forward SSR primer BNL3992

<400> SEQUENCE: 49

cagaagagga ggaggtggag 20

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: reverse SSR primer BNL3992

<400> SEQUENCE: 50

tgccaatgat ggaaaactca 20

<210> SEQ ID NO 51
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: forward SSR primer CIR280

<400> SEQUENCE: 51

actgcgttca ttacacc 17

<210> SEQ ID NO 52
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: reverse SSR primer CIR280

<400> SEQUENCE: 52

gcttcaccca ttcatc 16

<210> SEQ ID NO 53
<211> LENGTH: 165250
<212> TYPE: DNA
<213> ORGANISM: Gossypium hirsutum
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1104)..(1379)
<223> OTHER INFORMATION: Putative microsatellite region
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1105)..(1379)
<223> OTHER INFORMATION: Region with high homology with the DQ908392-
microsatellite region
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (3053)..(4915)
<223> OTHER INFORMATION: Putative uncharacterized protein F28J12.180
(Putative uncharacterized protein AT4g18520) (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (5208)..(5364)
<223> OTHER INFORMATION: exon1 from Putative uncharacterized protein
F7F23.4 (At1g36320/F7F23_4)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (5729)..(6147)
<223> OTHER INFORMATION: exon2 from Putative uncharacterized protein
F7F23.4 (At1g36320/F7F23_4)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (6475)..(6643)
<223> OTHER INFORMATION: exon3 from Putative uncharacterized protein
F7F23.4 (At1g36320/F7F23_4)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (6859)..(7103)
<223> OTHER INFORMATION: exon4 from Putative uncharacterized protein
F7F23.4 (At1g36320/F7F23_4)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (7526)..(7713)
<223> OTHER INFORMATION: exon5 from Putative uncharacterized protein
F7F23.4 (At1g36320/F7F23_4)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8004)..(8163)
<223> OTHER INFORMATION: exon6 from Putative uncharacterized protein
F7F23.4 (At1g36320/F7F23_4)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8164)..(8565)

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<223> OTHER INFORMATION: 3' Untranslated region from Putative
      uncharacterized protein F7F23.4 (At1g36320/F7F23_4)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (8636)..(9270)
<223> OTHER INFORMATION: ExonC from unknown protein (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (11837)..(12002)
<223> OTHER INFORMATION: Exon B from unknown protein (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (12184)..(12350)
<223> OTHER INFORMATION: Exon A from unknown protein (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (20866)..(21019)
<223> OTHER INFORMATION: 5' Untranslated region
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21020)..(21376)
<223> OTHER INFORMATION: exon1 from uncharacterized protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21020)..(21022)
<223> OTHER INFORMATION: start codon from DT563840
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (21503)..(22114)
<223> OTHER INFORMATION: exon2 from uncharacterized protein; DT563840
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (22112)..(22114)
<223> OTHER INFORMATION: stop codon from the DT563840-homology region
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (22115)..(22266)
<223> OTHER INFORMATION: 3' Untranslated region from the DT563840-
      homology region
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23354)..(23705)
<223> OTHER INFORMATION: 3' Untranslated region from the SHMT gene
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23706)..(23708)
<223> OTHER INFORMATION: TGA stop codon from SHMT (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (23706)..(23929)
<223> OTHER INFORMATION: Serine hydroxymethyltransferase (EC 2.1.2.1)
      (Serine methylase) (Glycine hydroxymethyltransferase) (SHMT)
      (Glycosylation-related protein 1) (Maternal effect lethal protein
      32)  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24355)..(24456)
<223> OTHER INFORMATION: Serine hydroxymethyltransferase (EC 2.1.2.1)
      (Serine methylase) (Glycine hydroxymethyltransferase) (SHMT)
      (Glycosylation-related protein 1) (Maternal effect lethal protein
      32)  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (25225)..(25633)
<223> OTHER INFORMATION: Serine hydroxymethyltransferase (EC 2.1.2.1)
      (Serine methylase) (Glycine hydroxymethyltransferase) (SHMT)
      (Glycosylation-related protein 1) (Maternal effect lethal protein
      32)  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (26181)..(27176)
<223> OTHER INFORMATION: Serine hydroxymethyltransferase (EC 2.1.2.1)
      (Serine methylase) (Glycine hydroxymethyltransferase) (SHMT)
      (Glycosylation-related protein 1) (Maternal effect lethal protein
      32)  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27174)..(27176)
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<223> OTHER INFORMATION: ATG start codon SHMT (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27386)..(27177)
<223> OTHER INFORMATION: 5' Untranslated region from the SHMT gene
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27729)..(28170)
<223> OTHER INFORMATION: Ex1-CERES41761278-homolog; also homologous to
      BE054702
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (27774)..(28329)
<223> OTHER INFORMATION: region with high homology with BE054702
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (29198)..(29833)
<223> OTHER INFORMATION: Ex2-CERES41761278-homology; also homologous to
      BE054702
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (30911)..(31136)
<223> OTHER INFORMATION: 3'UTR_GrpE/HSP-70; 3' Untranslated region
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31137)..(31139)
<223> OTHER INFORMATION: TAA stop codon (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (31137)..(31325)
<223> OTHER INFORMATION: ex3_GrpE/HSP-70; Protein grpE (HSP-70
      cofactor); putative heat shock protein  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32110)..(32583)
<223> OTHER INFORMATION: ex2_GrpE/HSP-70; Protein grpE (HSP-70
      cofactor); putative heat shock protein  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32817)..(33059)
<223> OTHER INFORMATION: ex1_GrpE/HSP-70; Protein grpE (HSP-70 cofactor)
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (33057)..(33059)
<223> OTHER INFORMATION: ATG start codon (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (33060)..(33234)
<223> OTHER INFORMATION: 5'UTR_GrpE/HSP-70; 5' Untranslated region
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (34773)..(35119)
<223> OTHER INFORMATION: ex3-ARF17; Putative exon3 from ARF17=
      determined by EST homology (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (36058)..(36815)
<223> OTHER INFORMATION: exon1 from the putative auxin response factor
      similar to At-ARF17 (At1g77850) (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (36099)..(36101)
<223> OTHER INFORMATION: TGA stop codon from the ARF17 gene (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (38053)..(39072)
<223> OTHER INFORMATION: exon2 from the putative auxin response factor
      similar to At-ARF17 (At1g77850) (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (39070)..(39072)
<223> OTHER INFORMATION: ATG start codon (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (39073)..(39113)
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<223> OTHER INFORMATION: 5' Untranslated region from the ARF17 gene=
      determined by EST homology (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (42998)..(43530)
<223> OTHER INFORMATION: repetitive region; similar to Gossypium
      raimondii repetitive sequences: DX405367, DX405119
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (44789)..(45407)
<223> OTHER INFORMATION: EST-homology= homology with ES817816-ES801532-
      DR460504
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (46515)..(46835)
<223> OTHER INFORMATION: ex4; DW507111; DW512136;uncharacterized protein
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (47486)..(47572)
<223> OTHER INFORMATION: ex3; DW512136;uncharacterized protein
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (51596)..(51868)
<223> OTHER INFORMATION: ex2-b; DR458828; uncharacterized protein
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (52127)..(52198)
<223> OTHER INFORMATION: ex2; DW512136;uncharacterized protein
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (52298)..(52711)
<223> OTHER INFORMATION: ex1; DW512136;uncharacterized protein
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (57253)..(57590)
<223> OTHER INFORMATION: 5'UTR-(eIF-5 1); 5' Untranslated region
      (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (57591)..(57593)
<223> OTHER INFORMATION: TGA stop codon  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (57591)..(58919)
<223> OTHER INFORMATION: Exon 1 from the Probable eukaryotic translation
      initiation factor 5-1 (eIF-5 1).  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (58917)..(58919)
<223> OTHER INFORMATION: ATG start codon (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (58920)..(59255)
<223> OTHER INFORMATION: 3'UTR-B (eIF-5 1); second  part of the
      3'Untranslated region form Probable eukaryotic translation
      initiation factor  5-1(eIF-5 1) - At1g77840 (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (60113)..(60244)
<223> OTHER INFORMATION: (complement) 3'UTR-A-(eIF-5 1); First part of
      the 3'Untranslated region form Probable eukaryotic translation
      initiation factor ? 5-1 (eIF-5 1) - At1g77840
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (66909)..(89556)
<223> OTHER INFORMATION: transposon region; region with high homology
      with transposable elements
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (76845)..(77564)
<223> OTHER INFORMATION: ORF-putative retrotransposon polyprotein; part
      of a retrotransposon-polyprotein (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
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<222> LOCATION: (88753)..(89556)
<223> OTHER INFORMATION: putative retrotransposon polyprotein; Putative
      polyprotein (Os05g0269800 protein)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (97467)..(97558)
<223> OTHER INFORMATION: ex1-Avr9; putative Avr9 elicitor response
      protein; galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (97467)..(97469)
<223> OTHER INFORMATION: putative start for the avr9 gene;
      galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (97716)..(97758)
<223> OTHER INFORMATION: ex2-Avr9; Putative Avr9 elicitor response
      protein; galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (97859)..(97917)
<223> OTHER INFORMATION: ex3-Avr9; Putative Avr9 elicitor response
      protein; galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (98038)..(98249)
<223> OTHER INFORMATION: ex3-b-Avr9; Putative Avr9 elicitor response
      protein; galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (98555)..(98627)
<223> OTHER INFORMATION: ex5-Avr9; Putative Avr9 elicitor response
      protein; galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (98722)..(98842)
<223> OTHER INFORMATION: ex6-Avr9; Putative Avr9 elicitor response
      protein; galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (98942)..(99029)
<223> OTHER INFORMATION: ex7-Avr9; Putative Avr9 elicitor response
      protein; galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (99143)..(99268)
<223> OTHER INFORMATION: ex8-Avr9; Putative Avr9 elicitor response
      protein; galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (100099)..(100101)
<223> OTHER INFORMATION: stop Avr9; TAA stop codon from avr9;
      galactosyltransferase family protein
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (100151)..(100517)
<223> OTHER INFORMATION: ex1-VPS9;Similarity to vacuolar protein
      sorting-associated protein VPS9 (Putative uncharacterized
      protein At3g19770).
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (100879)..(101000)
<223> OTHER INFORMATION: ex2-VPS9;Similarity to vacuolar protein
      sorting-associated protein VPS9 (Putative uncharacterized protein
      At3g19770).
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (101288)..(101559)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (101736)..(105725)
<223> OTHER INFORMATION: Putative retrotransposon  polyprotein;
      Retrotransposon protein, putative, Ty1-copia sub-class
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (106056)..(106327)
<223> OTHER INFORMATION: n is a, c, g, or t
<220> FEATURE:

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<221> NAME/KEY: misc_feature
<222> LOCATION: (108784)..(109261)
<223> OTHER INFORMATION: putative polyprotein  (complement)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (108785)..(108851)
<223> OTHER INFORMATION: EX-A_EST-homology1; homology with DW504110
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (108881)..(108930)
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<223> OTHER INFORMATION: ex8-HAT; exon8 from a putative histon acetyl
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<223> OTHER INFORMATION: ex4-HAT; exon4 from a putative histon acetyl
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<223> OTHER INFORMATION: ex3-HAT; exon3 from a putative histon acetyl
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<223> OTHER INFORMATION: CIR280-AJ567224; CIR280 homology region:
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<223> OTHER INFORMATION: ATG start codon (complement)
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<223> OTHER INFORMATION: ex1-MEKK1; Exon1 from a  putative Mitogen-
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<223> OTHER INFORMATION: retro-transposon region; region with high
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tgttatacca acttaatcaa aaattttatt aataaatatag acaaaatgat aaacgaattg 158700

ttagattaat taatttggat attgatccaa accaaacata agtatattat taaggaacgt 158760

agaattctaa taaacaattt tacatttcat gacccaacag tttgctttaa aggagtttat 158820

ttcaagccac tgtttgctag aaaaaatcta aaaaaaaaat gaaaatataa gtgagaatta 158880 tttcttcaaa aaaccaacaa attacacaaa tataatatga catttaaata tctaaattca 158940

aaacaaaaat taaaaaaata agtgggaatt attttttatt ttgagtaaag ccttatttaa 159000

aattcttgta caaattcaag caatattatc ctgttattta ttgatattat attttttttt 159060

aaaaaccctt aaaagcctac attttcttta ctccccactt gttctaatta aaactttcgg 159120

gtgaaatcaa gcctccaatg gcaaatatat gatcttctag agggaaccta gctatagact 159180

ttatttttta aaagaaaatt aaagttgtta aaaagtattt ttctttaata ttttcaaact 159240

tattaaattc atggacagta cttgatttag aaaccctaat caatcaagcc gattgagctt 159300

caacttagtt gatatcgtta ttgagttcaa gcacatttaa gcatattttt tttatataaa 159360

tattaaataa gaattttgaa aaaaaaaacc ttaatcaaga attgttgaaa aaaaattaac 159420

aaaaaaccca cttaatttgt agttacaaat atttgatgta tttaattaaa aatgctaaca 159480

ttttgaatat aatttgattt gaaggcatta aatatctcat gcgtcatcat cagttaataa 159540

taatataata tatttctaaa ttcaaaaatt gaaactttaa aaaacaatat ccgaaaccta 159600

aggcttgaaa gaaaaataaa ataaaataat tttaatttta attatgttta aaaatttgaa 159660

taaaaaactt gaaattcaaa accccaaatc tttaatttaa aataaaaaaa cttgaaactc 159720

aaaatttaaa cacagaaaga aaaacaaaat aattataatt aatatttaac tatctttaat 159780

acagttaata ttatttattt tcaagtcctt caattttttt ttcatttttt acaccaataa 159840

tgatgtaaaa caaatattaa tcatacatta aatttgtaaa ttagatctaa aaaataaaat 159900

atatactttt atattaataa atattaggtg cgatgataaa ttatattata tgttttaaaa 159960

aaaagaattc gagttcaaag attaaaagtg ttgattatta ggaaaacaac cgtgaactca 160020

aaaaaatatt aatttttata atttgaaaaa caaaaaaaaa atctgaataa tataaaaaac 160080

aataatactc ccatatgaca accaccacca taatgcttta attcaaaatg gtcccaactg 160140

gaaaacaaag aaagaaaaag ggcaggaaaa cagattaaat aaaatctttc cgtacaaaga 160200

tggatcgact gactgagttt ataaaatttt gttattattt attttagtaa acactgatat 160260

actttttagg cattcaattg caggagaggc aacgcccacc taccttcaac cccaaacaga 160320

tctcgtctct gttaaaattt gcaggcctac gcctagctat ctcctaaacg tttctcctct 160380

cacgtcttcc actgtttggt tctcgagaaa taaatcaatt tacaaattta atgccattca 160440

tcttcaacta tctttacctc tttcaaccca aaattttcaa tttattgcac cgtcctactc 160500

gtatcgtatg ttccccgtgt atatttcgct ctacgttttt tttttaatca ttttgtaata 160560

atttggtttt cctttttata tatatatatt tatttattta gggcttgaat ttttttactt 160620

catttctatg aattattttt aaatatatat atttttatgt ttattttatt gtctatattg 160680

gagatttgta aatatctctt ttaacaatgt tgtctcttta aactcgcgtt cttttcttgt 160740

taaaaaaata tatttaaatt atttttataa ttttaaatat aaaatatttt tacatcataa 160800

aaataaaaaa ttaataaaaa ataaaattcg ctccaattcg attttagtat ctacaacttt 160860

ttaatttaca tttcaaaaat aatccaaaca tcaattttca tttatttttc ttttttttca 160920

aaaaccctat atatggttat gaattgagct taaaataagc cttgtagata agtaagatga 160980

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gatttggaac actctctgat cttttaagtc aatctagaga ctttatcata aagaaatgaa 161100

agaatagagt gaaaaaagag actcaattaa aatttaaaaa ttatttatat taattattta 161160

aaaaatactt aaaatttata tttcatatac cacaatatta acaatgagtt gccgtgaatt 161220

attttattat atttacacta tcgttcaagt tgaatttttt ttaataccaa aataatacta 161280
acttggcatc gtagtaataa taacatgtaa aactaagaat aatttacatt taattctatt 161340
attaatttta ttataattta agtttatata tttattctat atatcataat attaattact 161400
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atgacatgac ggttctaatt tttccttctt tttttattgt atttgtggta ttgaggagaa 162420
gaaatatata gaaaatgaat aagttggtaa ttactaaatg tagcaaaacc cgaaaacatt 162480
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acccagggag ggaaaatatt tgtgaaactg gaagggataa acctataacc atatttcttt 162600
ttaatttatt gaccacattt ttggtttatt aaattgaatt atgaaaagag acagatcata 162660
tggaaaaagg tcccacttat caccagactc gtggctttgg gtctgcgcaa tcagacagta 162720
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gaaaagcatc actctactgt ttccttagcc cgactttgac ccttccccca ttttcaatta 162840
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gggcctgaac gatttcccct atatgggaga actgaagtgg cattttcaga ctagacacag 163740

gccagatgtt ttattccaat tagccttctg atcatgctct actcttcttc aatgaaaatt 163800

cttcctataa aatcccgaaa cctcttcgag tagagttttg gaccaacagc tgatattgaa 163860

gaaggatcag cttgtagtga tttgtaggca tgctccaatt tcttgctgat gtcgtagtct 163920

tgtaagatgt caatgatgcc aaagtataac actacttcat aaacttcgcc actatgtgaa 163980

aagagaccga ctccaccctg tgtatactga tcaaagtcgc ttcttcttga cattcgcact 164040

gctcttgctg gcatgtttgc tcctagccgt atcaatggtt tcctgccaaa cgaaaaatac 164100

atagaaaatt atttagaatt tctgttaagt tttctttccc tacagaatca caactcgcag 164160

aagaggcatc aatccacaac acataaaatt ctaactgcat actatcaaag tccatttcag 164220

atccaattcc ctaaagtatc tcaaatgcgt atataatttt caccaagagg gtgatgaaca 164280

aatggtatgt catatggtga cagtccaaag atcattagaa gggaaagaga ccttaaaata 164340

gaactagtga tatgcaccaa accatcgggt cacattctcc accattctca tccactgagt 164400

tcatgaatca tttcctagaa actagataaa tgggtaacaa aaaatcaagg caacccttga 164460

ttcttatcta gtaggatcat aaagggccac aattgctaca tgccccacca aaaaaaaaat 164520

ttgtcttctg aattatttcc acaaaatcac tcagacaaca caatcaacct tcaattattc 164580

catcgaatat gatcaagtat caacagttga ccgagaaatt taaaatttaa gcatcatcat 164640

ggaggagcct tgcctaattc cacatctaca ataaggaaat aactagaacc aaaagtatta 164700

tctaaggaag agagcaaaac atactggcca gctaaaatcc gatccatgtc ctgtagctca 164760

gcttcaagga atctacagcc acgcataaac ttttcattct gatatgaatc ctttttgcct 164820

gcatagggaa ggcatattca aatcccgatt cttatttcaa cagatattat ccattcaaat 164880

ttcatgagga atagtatggt ttctattaa cttacctgtg cgcaagagaa acggtgataa 164940

ccccatttta tcgcctctat tatcatcccg aaagtgtagt ccaaccaaaa gactataatc 165000

cataattctc tcagcctcca agaactcgca atctcgatca atttgcctat caccatgtag 165060

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gcttacttca taagctcttg gaaccaattc ctctggaggc gaaacacata attaagatcc 165180

aggtctttaa gggtagtggt ttcatcaatt tcctcttctg gcttatcagt tgagcggcca 165240

tgggaggatc 165250

<210> SEQ ID NO 54
<211> LENGTH: 1065
<212> TYPE: DNA
<213> ORGANISM: Gossypium barbadense
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (50)..(589)

<400> SEQUENCE: 54

aatatagtga aatatgggtc caagattttc tgggttttta atctaagca atg ctg ttt 58

Met Leu Phe
1

tta act caa ctc ctc tct cta aca gat ggc cgt gat att ggt gtt tgc 106
Leu Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys
5 10 15

tat ggt ttg aac ggc aac aat ctt cca tct cca gga gat gtt att aat 154
Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn

```
20                  25                  30                  35

ctt ttc aaa act agt ggc ata aac aat atc agg ctc tac cag cct tac      202
Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro Tyr
40                  45                  50

cct gaa gtg ctc gaa gca gca agg gga tcg gga ata tcc ctc tcg atg      250
Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met
55                  60                  65

agt acg aca aac gag gac ata caa agc ctc gca acg gat caa act cat      298
Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Thr His
70                  75                  80

caa agt gca gcc gat gca tgg gtt aac acc aac atc gtc cct tat aag      346
Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys
85                  90                  95

gaa gat gtt caa ttc agg ttc atc atc att ggg aat gaa gcc att cca      394
Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu Ala Ile Pro
100                 105                 110                 115

gga cag tca agc tct tac att cct ggt gcc atg aac aac ata atg aac      442
Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn Ile Met Asn
120                 125                 130

tcg ctc gcc tca ttt ggg cta ggc acg acg aag gtt acg acc gtg gtc      490
Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val
135                 140                 145

ccg atg aat gcc cta agt acc tcg tac cct cct tca gac ggc gct ttt      538
Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe
150                 155                 160

gga agc gat ata aca tcg atc atg act agt atc atg gcc att ctg gtt      586
Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Val
165                 170                 175

tga caggattcgc ccctcctgat caatgtgtac ccttattttg cctatgcctc           639

agaccccact catatttccc tcaactacgc cttgttcacc tcgaccgcac cggtggtggt    699

cgaccaaggc ttggaatact acaacctctt tgacggcata gtcgatgctt tcaatgccgc    759

cctagataag atcggcttcg gccaaattac tctcattgta gccgaaactg gatggccgac    819

cgccggtaac gagccttaca cgagtgtcgc gaacgctcaa acttataaca agaacttgtt    879

gaatcatgtg acgcagaaag ggctccgaa aagacctgaa tatataatgc cgacgttttt     939

cttcgagatg ttcaacgaga acttgaagca acccacagta gagcagatgt tcaacgagat    999

gttcaacgag aacttgaaat gttattgttg gctatttaaa tcttttgcca gagacgcttc   1059

atatag                                                              1065


<210> SEQ ID NO 55
<211> LENGTH: 179
<212> TYPE: PRT
<213> ORGANISM: Gossypium barbadense

<400> SEQUENCE: 55

Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile
1               5                   10                  15

Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp
20                  25                  30

Val Ile Asn Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr
35                  40                  45

Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser
50                  55                  60

Leu Ser Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp
65                  70                  75                  80
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Gln Thr His Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val
85                  90                  95

Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu
100                 105                 110

Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn
115                 120                 125

Ile Met Asn Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr
130                 135                 140

Thr Val Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp
145                 150                 155                 160

Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala
165                 170                 175

Ile Leu Val


<210> SEQ ID NO 56
<211> LENGTH: 1239
<212> TYPE: DNA
<213> ORGANISM: Gossypium darwinii
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (112)..(145)
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (258)..(760)

<400> SEQUENCE: 56

aagaaacgag caccagttat tgactttcct ttgtaaaaaa aaaaaaagtg ctgagatcaa     60

gaaatatagt gaaatatggg tccaagattt tctgggtttt taatctaagc a atg ctg     117

Met Leu
1

ttt tta act caa ctc ctc tct cta aca g gtaaaacaaa cttctctaca          165
Phe Leu Thr Gln Leu Leu Ser Leu Thr
5                   10

gtgattttac agtaaatatg gctttgaaaa atatacaaca aaacatttat cttcaatcca    225

ttttaattac tgatctacta tatatgttgc ag at  ggc cgt gat att ggt gtt      277

Asp Gly Arg Asp Ile Gly Val
15

tgc tat ggt ttg aac ggc aac aat ctt cca tct cca gga gat gtt att      325
Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile
20                  25                  30

aat ctt ttc aaa act agt ggc ata aac aat atc agg ctc tac cag cct      373
Asn Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Pro
35                  40                  45                  50

tac cct gaa gtg ctc gaa gca gca agg gga tcg gga ata tcc ctc tcg      421
Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser
55                  60                  65

atg agt acg aca aac gag gac ata caa agc ctc gca acg gat caa act      469
Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp Gln Thr
70                  75                  80

cat caa agt gca gcc gat gca tgg gtt aac acc aac atc gtc cct tat      517
His Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr
85                  90                  95

aag gaa gat gtt caa ttc agg ttc atc atc att ggg aat gaa gcc att      565
Lys Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu Ala Ile
100                 105                 110

cca gga cag tca agc tct tac att cct ggt gcc atg aac aac ata atg      613
Pro Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn Ile Met
115                 120                 125                 130
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aac tcg ctc gcc tca ttt ggg cta ggc acg acg aag gtt acg acc gtg      661
Asn Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val
135                 140                 145

gtc ccg atg aat gcc cta agt acc tcg tac cct cct tca gac ggc gct      709
Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala
150                 155                 160

ttt gga agc gat ata aca tcg atc atg act agt atc atg gcc att ctg      757
Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu
165                 170                 175

gtt tgacaggatt cgcccctcct gatcaatgtg taccсttatt ttgcctatgc           810
Val
ctcagacccc actcatattt ccctcaacta cgccttgttc acctcgaccg caccggtggt    870

ggtcgaccaa ggcttggaat actacaacct ctttgacggc atagtcgatg ctttcaatgc    930

cgccctagat aagatcggct tcggccaaat tactctcatt gtagccgaaa ctggatggcc    990

gaccgccggt aacgagcctt acacgagtgt cgcgaacgct caaacttata acaagaactt   1050

gttgaatcat gtgacgcaga aagggactcc gaaaagacct gaatatataa tgccgacgtt   1110

tttcttcgag atgttcaacg agaacttgaa gcaacccaca gttgagcaga tgttcaacga   1170

gatgttcaac gagaacttga aatgttattg ttggctattt aaatcttttg ccagagacgc   1230

ttcatatag                                                            1239


<210> SEQ ID NO 57
<211> LENGTH: 179
<212> TYPE: PRT
<213> ORGANISM: Gossypium darwinii

<400> SEQUENCE: 57

Met Leu Phe Leu Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile
1               5                   10                  15

Gly Val Cys Tyr Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp
20                  25                  30

Val Ile Asn Leu Phe Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr
35                  40                  45

Gln Pro Tyr Pro Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser
50                  55                  60

Leu Ser Met Ser Thr Thr Asn Glu Asp Ile Gln Ser Leu Ala Thr Asp
65                  70                  75                  80

Gln Thr His Gln Ser Ala Ala Asp Ala Trp Val Asn Thr Asn Ile Val
85                  90                  95

Pro Tyr Lys Glu Asp Val Gln Phe Arg Phe Ile Ile Ile Gly Asn Glu
100                 105                 110

Ala Ile Pro Gly Gln Ser Ser Ser Tyr Ile Pro Gly Ala Met Asn Asn
115                 120                 125

Ile Met Asn Ser Leu Ala Ser Phe Gly Leu Gly Thr Thr Lys Val Thr
130                 135                 140

Thr Val Val Pro Met Asn Ala Leu Ser Thr Ser Tyr Pro Pro Ser Asp
145                 150                 155                 160

Gly Ala Phe Gly Ser Asp Ile Thr Ser Ile Met Thr Ser Ile Met Ala
165                 170                 175

Ile Leu Val


<210> SEQ ID NO 58
<211> LENGTH: 1234
<212> TYPE: DNA
<213> ORGANISM: Gossypium darwinii
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<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (75)..(144)
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (239)..(1179)

<400> SEQUENCE: 58

aagaaacgag caccagttat tgacattcct ttgtaaaaaa aagaagaagc tgagatcaag      60

aaatatagtg aaat atg ggt cca aca ttt tct ggg ttt tta atc tca gca       110

Met Gly Pro Thr Phe Ser Gly Phe Leu Ile Ser Ala
1               5                   10

atg gtg ttt tta act caa ctc ctc tct cta aca g gtaaaacaaa              154
Met Val Phe Leu Thr Gln Leu Leu Ser Leu Thr
15                  20

cttctctaca gtgattttac ggtaagtatg gctttgaaaa atatacaaca aaacatttat     214

actgatctac catatatgtt gcag at  ggc cgt gat att ggt gtt tgc tat        264

Asp Gly Arg Asp Ile Gly Val Cys Tyr
25                  30

ggt ttg aac ggc aac aat ctt cca tct cca gga gat gtt att aat ctt       312
Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu
35                  40                  45

tac aaa act agt ggc ata aac aat atc agg ctc tac cag tct tac cct       360
Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Ser Tyr Pro
50                  55                  60

gaa gtg ctc gaa gca gca agg gga tcg gga ata tcc ctc tcg atg ggt       408
Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly
65                  70                  75                  80

ccg aga aac gag gac ata caa agc ctc gca aaa gat caa agt gca gcc       456
Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala
85                  90                  95

gat gca tgg gtt aac acc aac atc gtc cct tat aag gac gat gtt cag       504
Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln
100                 105                 110

ttc aag ttg atc act att ggg aat gaa gcc att tca gga caa tca agc       552
Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser
115                 120                 125

tct tac att cct gat gcc atg aac aac ata atg aac tcg ctc gcc tta       600
Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu
130                 135                 140

ttt ggg tta ggc acg acg aag gtt acg acc gtg gtc ccg atg aat gcc       648
Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
145                 150                 155                 160

cta agt acc tcg tac cct cct tca gac ggc gct ttt gga agc gat ata       696
Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
165                 170                 175

aca tcg atc atg act agt atc atg gcc att ctg gct gta cag gat tcg       744
Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser
180                 185                 190

ccc ctc ctg atc aat gtg tac cct tat ttt gcc tat gcc tca gac ccc       792
Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro
195                 200                 205

act cat att tcc ctc gat tac gcc ttg ttc acc tcg acc gca ccg gtg       840
Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val
210                 215                 220

gtg gtc gac caa ggc ttg gaa tac tac aac ctc ttt gac ggc atg gtc       888
Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val
225                 230                 235                 240
```

```
gat gct ttc aat gcc gcc cta gat aag atc ggc ttc ggc caa att act      936
Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile Thr
245                 250                 255

ctc att gta gcc gaa act gga tgg ccg acc gcc ggt aac gag cct tac      984
Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro Tyr
260                 265                 270

acg agt gtc gcg aac gct caa act tat aac aag aac ttg tta aat cat     1032
Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn His
275                 280                 285

gtg acg cag aag ggg act ccg aaa aga cct gaa tat ata atg ccg acg     1080
Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro Thr
290                 295                 300

ttt ttc ttc gag atg ttc aac gag gat ttg aag caa ccc aca gtt gag     1128
Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys Gln Pro Thr Val Glu
305                 310                 315                 320

cag aat ttc gga ttc ttc ttc ccc aat atg aac cct gtt tat cca ttt     1176
Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro Phe
325                 330                 335

tgg tgaagttgaa atgttgttgg ctatttaaat cttttgccag agacgcttca tatag    1234
Trp


<210> SEQ ID NO 59
<211> LENGTH: 337
<212> TYPE: PRT
<213> ORGANISM: Gossypium darwinii

<400> SEQUENCE: 59

Met Gly Pro Thr Phe Ser Gly Phe Leu Ile Ser Ala Met Val Phe Leu
1               5                   10                  15

Thr Gln Leu Leu Ser Leu Thr Asp Gly Arg Asp Ile Gly Val Cys Tyr
20                  25                  30

Gly Leu Asn Gly Asn Asn Leu Pro Ser Pro Gly Asp Val Ile Asn Leu
35                  40                  45

Tyr Lys Thr Ser Gly Ile Asn Asn Ile Arg Leu Tyr Gln Ser Tyr Pro
50                  55                  60

Glu Val Leu Glu Ala Ala Arg Gly Ser Gly Ile Ser Leu Ser Met Gly
65                  70                  75                  80

Pro Arg Asn Glu Asp Ile Gln Ser Leu Ala Lys Asp Gln Ser Ala Ala
85                  90                  95

Asp Ala Trp Val Asn Thr Asn Ile Val Pro Tyr Lys Asp Asp Val Gln
100                 105                 110

Phe Lys Leu Ile Thr Ile Gly Asn Glu Ala Ile Ser Gly Gln Ser Ser
115                 120                 125

Ser Tyr Ile Pro Asp Ala Met Asn Asn Ile Met Asn Ser Leu Ala Leu
130                 135                 140

Phe Gly Leu Gly Thr Thr Lys Val Thr Thr Val Val Pro Met Asn Ala
145                 150                 155                 160

Leu Ser Thr Ser Tyr Pro Pro Ser Asp Gly Ala Phe Gly Ser Asp Ile
165                 170                 175

Thr Ser Ile Met Thr Ser Ile Met Ala Ile Leu Ala Val Gln Asp Ser
180                 185                 190

Pro Leu Leu Ile Asn Val Tyr Pro Tyr Phe Ala Tyr Ala Ser Asp Pro
195                 200                 205

Thr His Ile Ser Leu Asp Tyr Ala Leu Phe Thr Ser Thr Ala Pro Val
210                 215                 220

Val Val Asp Gln Gly Leu Glu Tyr Tyr Asn Leu Phe Asp Gly Met Val
225                 230                 235                 240
```

Asp Ala Phe Asn Ala Ala Leu Asp Lys Ile Gly Phe Gly Gln Ile Thr
245 250 255

Leu Ile Val Ala Glu Thr Gly Trp Pro Thr Ala Gly Asn Glu Pro Tyr
260 265 270

Thr Ser Val Ala Asn Ala Gln Thr Tyr Asn Lys Asn Leu Leu Asn His
275 280 285

Val Thr Gln Lys Gly Thr Pro Lys Arg Pro Glu Tyr Ile Met Pro Thr
290 295 300

Phe Phe Phe Glu Met Phe Asn Glu Asp Leu Lys Gln Pro Thr Val Glu
305 310 315 320

Gln Asn Phe Gly Phe Phe Phe Pro Asn Met Asn Pro Val Tyr Pro Phe
325 330 335

Trp

<210> SEQ ID NO 60
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 60

atcctgtcaa accag 15

<210> SEQ ID NO 61
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 61

atcctgtcaa accag 15

<210> SEQ ID NO 62
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 62

gcttttggaa gcgatataac atcga 25

<210> SEQ ID NO 63
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 63

ggcataggca aaataagggt acaca 25

<210> SEQ ID NO 64
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 64

-continued

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aatatagtga aatatgggtc caag                                             24


<210> SEQ ID NO 65
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: oligonucleotide

<400> SEQUENCE: 65

aagaaacgag caccagttat tgac                                             24
```

The invention claimed is:

**1**. A non-naturally occurring *Gossypium* plant, and parts and progeny thereof, comprising at least one fiber strength allele which is derived from *Gossypium barbadense* of a fiber strength locus on chromosome A05 wherein said plant is a *Gossypium hirsutum* plant and wherein the fiber strength allele which is derived from *Gossypium barbadense* is located on chromosome A05 of *Gossypium barbadense:*

(a) between AFLP marker P5M50-M126.7 and SSR marker CIR280,

(b) between AFLP marker P5M50-M126.7 and SSR marker BNL3992,

(c) between AFLP marker P5M50-M126.7 and SSR marker CIR401c, or

(d) between SSR marker NAU861 or the GLUC1.1 gene and SSR marker CIR401c, said *Gossypium hirsutum* plant comprising a *Gossypium hirsutum* allele of at least one marker locus on chromosome A05 selected from the group consisting of CIR139a, BNL3029.A and NAU1042.A.

**2**. The plant of claim **1**, wherein said *Gossypium hirsutum* plant further comprises a *Gossypium hirsutum* allele of at least one marker locus on chromosome A05 selected from the group consisting of BNL0542, E43M49-M260.0, E31M48-M188.5, E43M53-M460.0, CIR294.A and BNL3995 which is derived from and specific for *Gossypium hirsutum.*

**3**. The plant of claim **1**, wherein the LOD peak of the fiber strength allele which is derived from *Gossypium barbadense* is located:

(a) at about 0 to 5 cM from SSR marker NAU861 or the GLUC1.1 gene, or

(b) at about 0 to 12 cM from SSR marker CIR401c.

**4**. The plant of claim **1**, wherein the fiber strength allele which is derived from *Gossypium barbadense* comprises a GLUC1.1 gene encoding a non-functional GLUC1.1 protein.

**5**. The plant of claim **1**, wherein the fiber strength allele which is derived from *Gossypium barbadense* comprises a GLUC1.1 gene characterised by the presence of a T nucleotide at a nucleotide position corresponding to nucleotide position 712 of SEQ ID NO: 5.

**6**. The plant of claim **1**, wherein the callose content of the fibers is increased compared to the callose content of the fibers of an equivalent *Gossypium* plant that does not comprise the at least one fiber strength allele which is derived from *Gossypium barbadense.*

**7**. The plant of claim **1**, wherein the strength of the fibers is increased compared to the strength of the fibers of an equivalent *Gossypium* plant that does not comprise the at least one fiber strength allele which is derived from *Gossypium barbadense.*

**8**. The plant of claim **7**, wherein the strength of the fibers is on average between about 1% and about 2% higher.

**9**. The plant of claim **7**, wherein the *Gossypium hirsutum* plant is homozygous for the fiber strength allele which is derived from *Gossypium barbadense.*

**10**. The plant of claim **3**, wherein the LOD peak of the fiber strength allele which is derived from *Gossypium barbadense* is located at about 4.008 cM from SSR marker NAU861 or the GLUC1.1 gene.

**11**. The plant of claim **3**, wherein the LOD peak of the fiber strength allele which is derived from *Gossypium barbadense* is located at about 10 cM from SSR marker CIR401c.

**12**. The plant of claim **3**, wherein the LOD peak of the fiber strength allele which is derived from *Gossypium barbadense* is located at about 10.52 cM from SSR marker CIR401c.

**13**. The plant of claim **9**, wherein the strength of the fibers is on average

(a) between about 5% and about 10% higher;

(b) between about 1.6 g/tex and about 3.3 g/tex higher; or

(c) between about 34.6 g/tex and about 36.3 g/tex.

* * * * *